The genetics of cutaneous sensitivity to ultraviolet radiation.

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Declaration

I hereby declare that

i)this thesis has been composed entirely by myself,

ii)that the work presented is my own, except where otherwise stated

iii)no part of this work has been submitted for any other degree or professional qualification.

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Abstract

Ultraviolet radiation (UVR) is present in the sunlight that reaches the Earth, and can also be artificially produced. Artificial sources of UVR can be used therapeutically, for the treatment of skin disease such as psoriasis. UVR is a source of DNA damage, and the major causative agent for the development of skin cancers. UV-induced DNA damage can be repaired by a number of DNA repair pathways, principally the nucleotide excision repair (NER) pathway and the base excision repair pathway (BER), preventing DNA lesions from becoming incorporated into the genome.

Defects in the genes involved with NER lead to three, rare, recessive syndromes, Xeroderma pigmentosum (XP), Cockayne's Syndrome (CS) and Trichothiodystrophy (TTD). Individuals affected with any of these syndromes have varying degrees of photosensitivity, with XP patients also having a greater than 1000 fold increased risk of skin cancer. As UVR is one of the most frequently exposed to sources of DNA damage, and also used as a therapeutic reagent, it would be useful to have a genetic marker which might predict an individual's response to UVR.

Polymorphisms in the genes involved with the NER, BER and other repair pathways will be examined in this thesis, to determine if any are associated with sensitivity to UVR. Sensitivity to UVR will be determined as the level of erythema induced by an incremental range of UV doses in two independent study groups, of 74 and 31 individuals. Erythema will be measured using reflectance spectrophotometry, which is an analytical measure, rather than the widely used minimal erythemal dose (MED).

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Abbreviations

µl	microlitre
6-4PP	6-pyrimidine-4-pyrimidone photoproduct
8-MOP	8-methoxypsoralen
aa	amino acid
Arg	Arginine
Asp	Asparagine
BCC	basal cell carcinoma
BER	base excision repair
CA	chloroform, iso-amyl alcohol solution
CI	confidence index
CKM	muscle creatine kinase
cm	centimetres
CPD	cyclobutane pyrimidine dimer
CS	Cockayne's Syndrome
DNA	Deoxyribonucleic acid
DSB	double strand break
ERCC	excision repair cross complementing
ExoI	Exonuclease I
FEN1	flap endonuclease
GGR	global genome repair
Gln	Glutamine
GSH	glutathione
GST	glutathione S-transferase
His	Histidine
HNPCC	hereditary non-polyposis colon cancer
HPV	human papilloma virus
HR	homologous recombination
J	joules
Kb	kilobase
Lys	Lysine

MED	minimal erythemal dose
ml	millilitre
MMR	mismatch repair
N	number
NER	nucleotide excision repair
NHEJ	non-homologous end joining
nm	nanometres
NMSC	non-melanoma skin cancer
nt	nucleotide
OMIM	Online Mendelian Inheritance in Man
OR	odds ratio
PARP	Poly (ADP-ribose) polymerase
PBS	phosphate buffer saline
PCA	phenol, chloroform, iso-amyl alcohol solution
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
Pro	Proline
PUVA	photochemotherapy
RFLP	restriction fragment length polymorphism
RNA	Ribonucleic acid
ROS	reactive oxygen species
RPA	replication protein A
SAP	shrimp alkaline phosphatase
SCC	squamous cell carcinoma
SCCHN	squamous cell carcinoma of the head and neck
TCR	transcription coupled repair
Tg	thymine glycol
Trp	Tryphtophan
TTD	Trichothiodystrophy
UVA	ultraviolet radiation A
UVB	ultraviolet radiation B
UVC	ultraviolet radiation C
UVR	Ultraviolet radiation
XP	Xeroderma pigmentosum

XRCC	X-ray repair, complementing defective in Chinese hamster

Foreword

The sunlight that reaches the Earth is part of a continuous spectrum of electromagnetic radiation. Of great biological and medical interest is one component of this electromagnetic spectrum, ultraviolet radiation (UVR), which ranges from 100-400 nanometres (nm). Other components of the electromagnetic spectrum are radiowaves, microwaves, infrared, visible light (400-700nm), X-rays and gamma radiation. The properties of any particular component of the spectrum are characterised by the wavelength of the radiation.

Ultraviolet radiation is subdivided into three regions, UVA, UVB and UVC. UVA has the longest wavelengths, from 315 to 400nm, UVB 280-315nm and UVC 100-280nm. These regions are usually further defined by dermatological photobiologists as UVA 320-400nm, UVB 320-290nm, and UVC as 290-200nm. Little UVC radiation reaches the surface of the earth, due to it being blocked by the stratospheric ozone layer, exposure to UVC is therefore mainly through man-made sources, such as germicidal lamps. UVA and UVB both reach the Earth's surface, and can be absorbed by the skin. In summer, in the UK, UVB (when defined as 280-315 nm) comprises approximately 3.5% of the UVR that reaches the Earth's surface, while UVA comprises the remaining 96.5%.

Beneficial Effects of UVR

Phototherapy

UVR is commonly used as a treatment for psoriasis in Dermatology clinics. Psoriasis is a chronic, relapsing skin disease with variable clinical features. The most common type is psoriasis vulgaris. Circular plaques are predominant on the elbows, knees and lower back, whereas erupting guttate lesions are often confined to the trunk and proximal extremities. Psoriasis is a disease universal in its occurrence. The worldwide incidence varies, in the USA affecting approximately 2% of the population, in the UK around 1.6%, 0.97% in South America, and 2.8% in the Faeroe Islands. The higher incidence of psoriasis in the Faeroe Islands could be partly due to a Founder effect, leading to relatively little genetic variation, but also due to its latitude at 61° north. Sunlight is

beneficial in the treatment of most cases of psoriasis. Psoriasis is due to excessive, but controlled, cellular proliferation and inflammation, occurring within 0.2mm of the surface of the skin. UVA and UVB are both used in different treatment regimes to "clear" psoriatic plaques. UVA is commonly used during photochemotherapy (PUVA). Patients orally ingest a potent photosensitiser, such as 8-methoxypsoralen (8-MOP) and are then exposed to variable doses of UVA. Psoralens such as 8-MOP intercalate with DNA, and with the energy from UVA, co-valently cross link nucleic acids with opposing strands of duplex regions of DNA. The formation of these cross-linking photoadducts leads to the irreversible photo-inhibition of DNA synthesis and mitosis (Pohl *et al*, 1979). This reaction is thought to be important in the hyperproliferative psoriatic epidermis. A typical course to clear psoriasis is 19-25 treatments. The long-term effects of PUVA include photoageing, but also photocarcinogenesis. Patients who have received PUVA treatment have a dose-related increased frequency of squamous cell carcinoma (SCC) (Stern *et al*, 1984). Patients who have had over 250 PUVA treatments are especially at risk of malignant melanoma (Stern *et al*, 1997).

UVB is also used in the treatment of psoriasis. It is thought to be efficient at clearing plaques through its immune suppression activities, depleting Langerhans cells, decreasing leukocyte adhesion to the microvasculature, leading to the depletion of intraepidermal T cells, and the induction of IL-10 production from macrophages, which acts as an anti-inflammatory mediator (Cai *et al*, 1996; Krueger *et al*, 1995; Meunier *et al*, 1995 and Kang *et al*, 1994).

Photosynthesis of Vitamin D

Vitamin D is essential for health, promoting the absorption of calcium and phosphate from food. It is essential in the formation of bones and teeth. Deficiencies of vitamin D leads to a failure of the bones to grow, and causes rickets in children and osteomalacia in adults. UVR photosynthesises 7-dehydrocholesterol into vitamin D3. 7-dehydrocholesterol is present in the epidermis of the skin, and absorbs UVR less than 320nm. Upon absorbance of UVR, this converts into the vitamin D precursor, previtamin

D3. Previtamin D3 then isomerises to become vitamin D, binding to binding proteins present in the capillaries. Although in most industrial countries the level of vitamin D present in food is enough to satisfy requirements for everyday health, it has been suggested that recent sun awareness campaigns have resulted in vitamin D deficiencies in some individuals, particularly the elderly, in whom levels of 7-dehydrocholesterol in the skin decrease with age. In its role in the photosynthesis of vitamin D, UVR has a beneficial effect on our health. Some studies have also suggested vitamin D may play a role in the regulation of the cell cycle, and might have an effect in reducing cancer of the prostate (Konety *et al*, 1999; Getzenberg *et al*, 1997) and of the breast (Lowe *et al*, 2003; O'Kelly and Koeffler, 2003).

Erythema

The main acute clinical effects of UVR on normal skin are UV-induced erythema, sometimes referred to as sunburn inflammation, and tanning. Chronic exposure of the skin to UVR leads to photoageing, and carcinogenesis. In general, the intensity of erythema is determined by the amount of radiation the skin receives. Low doses over a longer period of time will lead to the same level of erythema as a higher dose over a shorter period of time. A method of determining skin sensitivity to UVR is by determining the amount of UVR required to induce visible erythema after 24 hours, the minimal erythemal dose (MED). This can be just perceptible erythema (MEDjp) or clearly defined erythema with distinct margins. Erythema is the most conspicuous acute response to UVR, and can vary greatly between individuals, and between body sites within individuals. The MED is routinely used as an end-point for the assessment of erythema in both clinical practise and research studies. However, it is a subjective measure, and is therefore open to errors of judgement. The MED is based on an assessment of a binary outcome, whether a particular degree of erythema occurs following exposure to a graded series of doses of UV (Farr and Diffey, 1984, 1986). The particular degree of erythema chosen as the endpoint varies between researchers, as mentioned above this can include just perceptible erythema or erythema with distinct margins. Error can be introduced when determining the MED as it is dependent upon the

human eye and definition of erythema that is just perceptible or that with defined margins can vary between individuals. As the MED is a threshold measurement, the exact value of the MED is dependent on the dose increment chosen for the series of irradiation (Diffey and Farr, 1989; Lock-Andersen and Wulf, 1996).

An action spectrum demonstrates the relative photobiological efficiency at different wavelengths, and can be used to identify chromophores or to calculate the relative importance of difference wavelengths under actual environmental conditions. The action spectrum for erythema can be seen in the figure below.

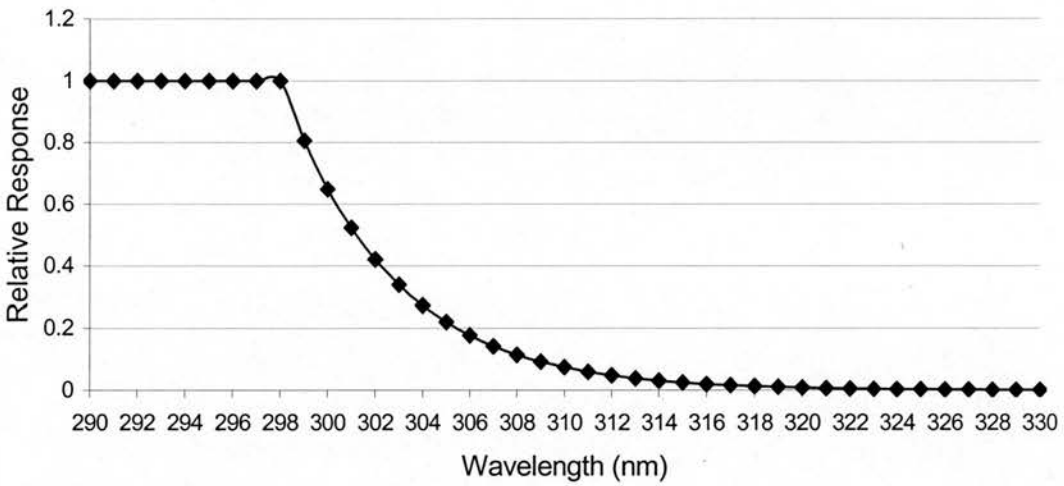


Figure 1, erythemal action spectrum

In animals, UVB-induced erythema peaks between 6 and 24 hours, depending upon UVR dose (Macher *et al*, 1969). UVA also induces erythema, often causing an immediate erythemal response, which is followed by a second erythema as with UVB. Both UVA and UVB-induced erythema can be prolonged in fair-skinned individuals. Some individuals with the UV-sensitive disorder Xeroderma pigmentosum (described later) also show prolonged UV-induced erythemal responses, lasting for several weeks. Chromophores within the epidermis absorb UVR, changing the skin biology. Chromophores within the epidermis are DNA, proteins, urocanic acid and melanin precursors. Nuclear DNA is the most well characterised chromophore for UVB. Young *et*

al (1998) determined action spectra for thymine dimers, a type of cyclobutane dimer, and UV-induced erythema. The epidermal dimer action spectra was analysed and compared with erythema action spectra from the same UVR sources and group of volunteers. Comparisons between the two suggest the DNA is the major chromophore for erythema at 280-340nm.

Skin Cancer

The link between UVR and skin cancer was first described over 100 years ago, when, in 1894, Unna described that sun exposed skin sites in sailors were likely to develop skin cancer. Dubrevilh (1896) noted an increased frequency of keratoses and skin cancer cases in vineyard workers compared with their mainly indoor-working city dwelling neighbours in the Bordeaux region of France. The development of artificial sources of UVR led to early mouse experiments which demonstrated that mice irradiated daily with UVR from a mercury arc developed skin cancers unlike mice of the same strain who were not exposed to UVR (Findlay, 1928). It is now well established that repeated exposure to sun light, or artificial sources of UVR, such as tanning lamps, over a period of years can lead to chronic degenerative changes in human skin – skin ageing, premalignant and malignant skin lesions. Skin cancer in humans can be classified into one of two categories, non-melanoma skin cancer (NMSC) and malignant melanoma. NMSC are predominantly basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Together, BCC and SCC are the most common cancer in humans (Glass and Hoover, 1989), but fortunately are also one of the most easily and successfully treated cancers in man. In contrast, malignant melanomas are less common than NMSC, although their incidence in increasing, but are potentially much more dangerous. Skin cancer is a growing problem in the UK. Data from the UK cancer registries shows that 70,038 cases of skin cancer were registered in 2001 (Cancer Research UK, 2004). Of these, the vast majority, over 62,700, were cases of NMSC. It is known that not all cases of NMSC are registered; therefore there the true number of NMSC cases will be higher. More than 7,300 cases of malignant melanoma were diagnosed in 2001. In 2003, 2280 people died from skin cancer in the UK, of which 1770 were due to malignant melanoma,

demonstrating the seriousness of this cancer. Within the last ten years, the problem of malignant melanoma has dramatically increased; with the increase in incidence the largest of all incidence rates compared with other major cancers (Cancer Research UK, 2002).

Tanenbaum *et al* (1976) found that individuals with a prolonged erythemal response or a more intense erythemal response to UVR were at an increased risk of developing skin cancer. Mutations in the tumour suppressor gene p53 are one of the most common in a great variety of human cancers. Studies by Daya-Grosjean *et al* (1995) and Ziegler *et al* (1993) suggested that UVR-induced mutations of p53 play a key role in the initiation of NMSCs. Tandem dipyrimidine mutations in p53 are rare in internal cancers, with a frequency of less than 1%, but occur in 14% of NMSC in the general population, and in 53% of NMSCs in Xeroderma pigmentosum patients. 61% of p53 base substitutions occur opposite dipyrimidine sequences in internal tumours, compared with an increased frequency of over 90% in NMSC (Daya-Grosjean *et al*, 1995).

UVR and DNA

UV-induced photoproducts

UVA and UVB are both able to mutate DNA. DNA is efficient at absorbing UVR, with maximal absorption in the UVC region at around 260nm. There is also significant absorption in the solar UVB region, as well as some absorption in the UVA region (Sutherland and Griffin, 1981). UVB is therefore much more efficient at causing biological damage than UVA, contributing around 80% towards UV-induced DNA damage, while UVA contributes towards only 20% of UVR-induced DNA damage. While UVB-induced DNA damage is direct, UVA-mediated damage occurs indirectly, through absorption of UVR through exogenous sensitisers, which generate reactive oxygen species (ROS). These oxygen radicals can in turn lead to breaks in the DNA, which will ultimately cause the accumulation of mutations if not repaired. Cadet and colleagues (2000) found that the oxygen radicals formed by UVA radiation target the guanine base of DNA, giving rise to the mutagenic species, 8-hydroxyguanine. Of the

UVR that reaches the earth, UVB is the most damaging component to animals, including humans. UVB can penetrate the skin's epidermis, leading to erythema and burning at the cutaneous level, but also inducing DNA damage at a cellular level, such as cyclobutane pyrimidine dimers (CPD), and 6-pyrimidine-4-pyrimidone photoproducts (6-4PPs).

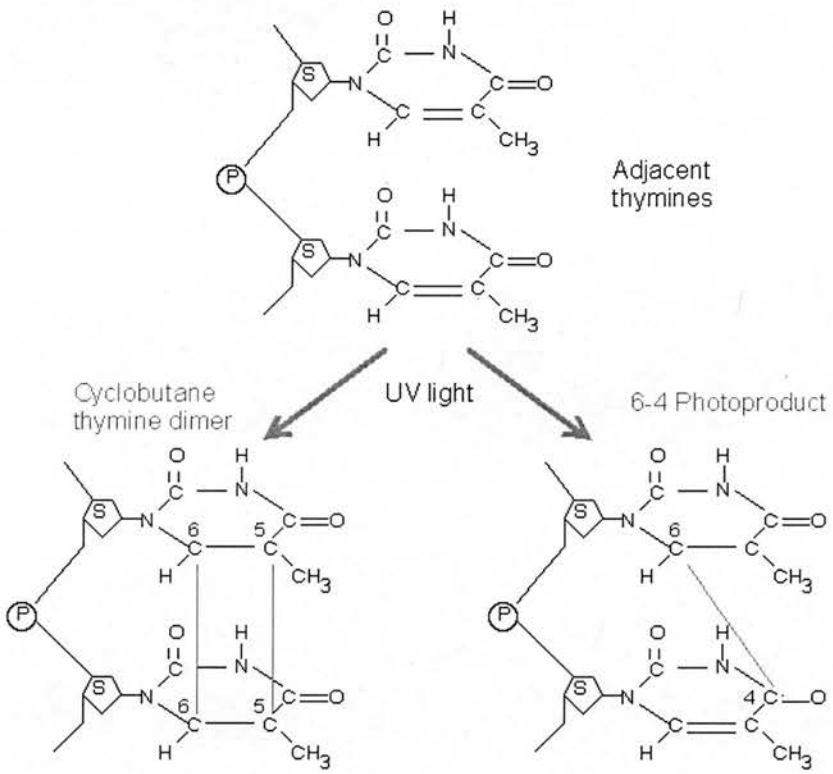


Figure 2, Pyrimidine dimer induced by UVR. This figure uses thymine as an example. Cytosine may form a similar dimer.

CPDs are the major class of lesion produced by UVB radiation; 6-4PPs are the second most common, with CPDs occurring 5-10 times more often (Eveno *et al*, 1995). CPDs and 6-4PPs are most common where there are strings of pyrimidine bases in the DNA. 6-4PPs are formed between the 5' six position and the 3' four position of two adjacent pyrimidines, most often between TC and CC residues. UVB-induced DNA lesions are extremely common – Ichihasi and colleagues (1998) found that exposure to sunlight for approximately 60 minutes at noon during summer in Kobe, Japan (latitude 34°N) led to

the development of approximately 100,000 CPDs per cell. Left unrepaired this DNA damage will result in the accumulation of DNA mutations, ultimately leading to carcinogenesis. Ruven *et al* (1993) investigated the rate of repair of CPDs in the mouse, and found that 60% of CPD repair activity in actively transcribed genes occurred within the first four hours after exposure to UVR, with no repair in non transcribed strands, the same group showed preferential repair of mutations in the p53 gene in the transcribed strand of DNA in mouse skin, with 80% of lesions repaired after 24 hours, with no such repair being observed in non-transcribed strands of the same gene (Ruven *et al*, 1994). Mutations induced by both CPDs and 6-4PPs include C→T or CC→TT transitions, the latter being a hallmark of UV damage. The mechanism that repairs these UVR-induced lesions is known as Nucleotide Excision Repair (NER). As well as CPDs and (6-4)PPs, UVR also induces other types of lesions, such as single-strand breaks in DNA. As mentioned above, UVA, and visible light, induce DNA damage indirectly through the generation of reactive oxygen species, short-lived, highly reactive molecules, which produce single-stranded breaks in the DNA, DNA-protein crosslinks, and altered DNA bases. Altered bases, in particular 8-hydroxyguanine, are a more frequent product of reactive oxygen species than single-strand breaks or DNA-protein crosslinks (Tchou *et al*, 1991).

Reactive oxygen species

Reactive oxygen species (ROS) are a family of oxygen-based free radicals that contain, or are capable of producing, an unpaired electron. The major intracellular source of oxygen radicals is probably leakage associated with the reduction of oxygen to water during mitochondrial respiration. These products are singlet oxygen, peroxide radicals ($\cdot O_2$), hydrogen peroxide (H_2O_2) and hydroxyl peroxide ($\cdot OH$). Extracellular sources of reactive oxygen species include ionizing radiation, and UVR, particularly at 320 to 380nm (Tyrell, 1991).

At low levels, the superoxide ion, hydroxyl radicals, hydrogen peroxide and molecular oxygen are involved with many cellular processes, such as cell proliferation, apoptosis and cellular differentiation. However, overproduction, or the inefficient removal, of ROS

generates oxidative stress, which has a negative impact on cellular integrity. The ·OH radical is the most directly damaging to DNA of these components. The ·OH radical can damage DNA directly by attacking the double bond of thymine at C-5 or C-6, and, to a lesser extent, can abstract hydrogen from the methyl group (Jovanovic and Simic, 1986; Simic, 1994). This intermediate can react with O_2 to yield thymine glycol (Demple and Linn, 1982) which appears to block DNA replication (Ide *et al*, 1985; Clark and Beardsley, 1987; Clark *et al*, 1987). The ·OH radical can also generate 8-hydroxyguanine, due to the addition of the ·OH group to C-8 of guanine (Jovanovic and Smith, 1989; Kasai and Nishimura, 1991). 8-hydroxyguanine is a mispairing lesion during DNA replication, which if not repaired will result in DNA damage (Kuchino *et al*, 1987; Shubutani *et al*, 1991; Cheng *et al*, 1992). Antioxidants help to delay or prevent ROS-induced damage on cells, by reducing local oxygen concentrations, impairing chain initiation reactions. Antioxidants include β-carotene, ascorbate, uric acid and glutathione (GSH). GSH is an important water-phase antioxidant, which is conjugated by the Glutathione S-transferase (GST) enzymes. GSH is involved in the recycling of oxidised antioxidants and serves as a scavenger of O_2 and OH (Rice-Evans and Burdon, 1994).

The effect of anthralin on DNA

Anthralin is a substance commonly used in the treatment of psoriasis (see Kemeny *et al*, 1990, for a review of its mode of action). The extract of the legume tree *Andira araroba* (Goa powder) was first seen to be beneficial in the treatment of psoriasis by the English dermatologist Squire. Liebermann and Seidler determined in 1878 that the active component in Goa powder was the anthrone-derivative chrysarobin, 1,8-dihdroxy-3-methyl-9-anthrone. Based on this, the first synthetic psoriasis treatment, chrysarobin, 1,8-dihydroxy-3-methyl-9-anthrone, was developed in Germany, and later named anthralin (also known as dithranol). Anthralin acts via several mechanisms, which are not fully understood, but include the inhibition of cell proliferation (Kemeny *et al*, 1990) and the suppression of the immune system (Anderson *et al*, 1987). The antiproliferative effect is thought to be due in some part to an effect on DNA duplication and repair (Lowe and Breeding, 1981). Anthralin is highly reactive and is readily oxidised by light and oxygen

(Hsieh and Acosta, 1991). Its oxidised metabolites lead to the formation of reactive oxygen species (ROS) that are suggested to play an important role in the mode of action of anthralin (Finnen *et al*, 1984). Anthralin has been shown to generate ROS in several studies (Bruce *et al*, 1987, Muller *et al*, 1990, Halmekoski *et al*, 1982). Müller *et al* (1987) demonstrated that anthralin generates superoxide anion particles by the measurement of the auto-oxidation of anthralin by the reduction of the superoxide scavenger Nitro blue tetrazolium. The consequences of oxidative stress are repaired by the DNA repair pathways base excision repair (BER) and nucleotide excision repair (NER) (see later for details of these pathways). It is possible that polymorphisms in genes involved in these pathways will affect the ability of the repair system to deal with the oxidative stress generated, resulting in increased sensitivity to anthralin.

DNA Repair Pathways

Mammalian cells can make use of a variety of repair pathways, many of which overlap and share components. DNA repair pathways show remarkable evolutionary conservation, demonstrating their great importance. Lindahl (1993) estimates that several thousand DNA alterations occur per cell per day. If efficient DNA repair pathways did not exist, our DNA would be destroyed over our lifetime. NER, along with other repair pathways, will be considered in some detail in this introduction.

Direct Reversal of Damage – Photoreactivation of DNA

Photoreactivation is a process that reverses the most common DNA lesions that are induced by UVR, the CPD, in many prokaryotes and eukaryotes. It is not, however, found above the evolutionary level of marsupials in mammals (Li *et al*, 1993; Yasui *et al*, 1994). The enzymes that catalyse this reaction are termed photolyases, and are most widely studied in *E.coli*. In *E.coli*, under normal conditions, there are only 10-20 molecules of DNA photolyase. The photolyase has two chromophore co-factors, which absorb light. Upon absorption of a single photon by one chromophore, its excitation energy is transferred to the second chromophore. Excitation of the second chromophore

leads to electron transfer to the CPD. This electron transfer causes the cyclobutane dimer to revert to a monomeric state.

Double Strand Break Repair

Double stand breaks (DSBs) in DNA can occur for a number of reasons, and can be environmental or spontaneous. Environmental causes of DSBs include ionising radiation, free radicals and mechanical stress. DSBs can also occur due to endonuclease activity in the DNA repair process and due to recombinational events, such as meiosis or V(D)J recombination, which are normal cellular processes. Leaving DSBs unrepaired can lead to DNA mutations or rearrangement of chromosomes, which prevents the correct segregation of the genome to daughter cells.

In mammals, the main pathway for the repair of DSBs is Non-Homologous End Joining (NHEJ). In lower eukaryotes, such as yeast, homologous recombination (HR) carries out DSB repair.

During NHEJ, an extended region of homology between the DNA strands at the repair site is not needed. This enables NHEJ to take place when there is as little as a single complementary base pair between the overhanging ends of DNA. Roth and Wilson (1986) determined that NHEJ is required to rejoin specific DSBs produced during V(D)J recombination. Mammalian cells deficient in NHEJ are immunodeficient, due to their inability to complete V(D)J recombination, and are hypersensitive to ionising radiation which induced DSBs (Baumann *et al*, 1998).

NHEJ in eukaryotes has been analysed through *in vitro* studies, and the proteins required have been shown to be DNA Ku, DNA-PK(cs), XRCC4 and DNA ligase IV, which either bind the ends of linear DNA directly, or through associations with other proteins. In yeast, Rad50, Mre11/Xrs2 and the Sir proteins 2, 3 and 4 are also required. DNA-PK(cs) is the catalytic subunit of DNA protein kinase, which regulates kinase activity through its association with the DNA. DNA-PK(cs) is recruited to the site of the DSB by the DNA Ku protein, a heterodimer of Ku70 and Ku80, which when all together comprise the whole DNA protein kinase complex. XRCC4 and DNA ligase IV form a complex, which ligates one strand of the DNA with minimal base pairing to the anti-parallel strand. If the

repair process needs to remove any overhanging DNA at the site of the DSB, or resynthesise a missing gap, nuclease or polymerase is required. Overhanging DNA sequences can occur as the DNA is being aligned according to regions of microhomology. The polarity of the DNA strand determines which type of nuclease is required, Wu *et al* (1999) and Lieber (1999) proposed that the 5' nucleases FEN1 and EXO1, and the 3' nuclease MEW11 might be likely candidates. An alignment protein, which was proposed by King *et al* (1994) to be DNA polymerase, is thought to be required to hold the strands in place while any modifications are being carried out before the ligation of both strands by DNA ligase IV. Murine knockouts of DNA ligase IV and XRCC4 are embryonic lethals, due to their inability to carry out NHEJ (Lieber, 1999). Small deletions in the DNA that extend in both directions from the DSB site and end within the region of microhomology is a common result of NHEJ, and typically results in regions of short repeats around the DSB.

Homologous Recombination

Homologous Recombination (HR) gives faithful restoration of a sequence at the point of the DSB. The process requires a non-damaged template for the restoration of the damaged or missing sequence; the homologous chromosome to the damaged DNA. The 5' strand ends of the DSB are digested by a nuclease, believed to be encoded from the Mre11/RAD50/NbsI (MRN) protein, to yield 3' single stranded overhanging tails (D'Amours and Jackson, 2002; Tauhi *et al*, 2002). The RAD52 group of genes then encode a protein that binds to the 3' single strand DNA ends in S.cerevisiae (Van Dyck *et al*, 1999; Stasiak *et al*, 2000). This complex targets the template for repair, the homologous intact duplex DNA on the undamaged chromosome. Single strand exchanges occur, leading to the formation of Holliday junctions, joint molecules between the damaged strand and the template strand. Synthesis of the missing DNA is initiated, and the Holliday junctions are then resolved (Szostak *et al*, 1983).

Base Excision Repair

Base Excision Repair (BER) removes damaged or inappropriate DNA bases, which can be small DNA adducts, fragmented bases, oxidised and reduced bases and apurinic/apyrimidinic (AP) sites. This DNA damage can come from a number of sources, free radicals resulting from normal metabolic processes or ionising radiation can give rise to reduced or fragmented bases, spontaneous hydrolysis or oxidative damage can give rise to AP sites. There are two subpathways of BER, which contain some common proteins; short-patch BER, which repairs single bases, and long-patch BER, which repairs strands of bases from 2-10nt long.

Short-patch BER

In the short patch BER pathway, recognition of a damaged base and initiation of BER is carried out by a DNA glycolyase specific to that base, for instance, the base uracil, which is inappropriate for DNA, is recognised by uracil-DNA glycosylase, while methylated adenine is recognised by 3-methyladenine DNA glycosylase. Human glycosylases have structural and functional homology to bacterial glycosylases, for example, the human glycosylase NTH1, which has specificity for a variety of oxidised pyrimidines, has been shown to be the homologue of the bacterial endonuclease III (Aspinwall *et al*, 1997). The N-glycosylic bond between the base and the deoxyribose-phosphate backbone is then hydrolysed, releasing the base (Dianov and Lindahl, 1994; Frosina *et al*, 1996; Lindahl, 1995). Paradoxically, this then forms an AP site, which in itself is a type of DNA damage. REF1, an AP endonuclease, makes an incision 5' to the AP site by hydrolysis of the bond in the phosphodiester backbone (Demple and Harrison, 1994; Sancar and Sancar, 1998; Wallace, 1998). The AP endonuclease leaves a strand break with a 5' terminal deoxyribose-phosphate moiety (dRP), which must be removed before repair synthesis can occur. In short-patch BER, the deoxyribose 5' phosphatase activity of the DNA polymerase β removes the dRP, and DNA polymerase β can then synthesise new DNA. DNA ligase III and XRCC1 are responsible for the ligation of the newly synthesised DNA to the damaged strand. DNA polymerase β is thought to be regulated by the tumour suppressor p53, thus p53 can affect BER. Zhou *et al* (2001) determined that cells that lacked p53 were deficient in BER.

Long-patch BER

During long-patch BER, the dRP moiety, along with several downstream nucleotides, is removed by flap endonuclease I (FEN1). The damaged bases being repaired are displaced, creating a 5' terminal overhang. FEN1 binds to and moves along the overhanging region from the 5' end, until it reaches the cleavage site. Here it makes an incision that releases the overhanging region. PCNA interacts with FEN1, enhancing its ability to bind and cleave. FEN 1 is displaced from PCNA by p21, and released from the replication fork for the removal of the dRP. DNA polymerases δ and/or ε are utilised in the place of polymerase β in long-patch BER, and ligation is performed by DNA ligase I (Fortini *et al*, 2003; Klungland and Lindahl, 1997).

Defects in key proteins of the BER process, such as AP endonuclease or DNA polymerase β, lead to embryonic lethal phenotypes in the mouse, indicating the repair of endogenous DNA lesions is essential during development (Wood and Thompson, 1997).

Mismatch Repair

Mismatched bases can be introduced into DNA during DNA synthesis or recombination events. Bases can be incorporated during synthesis if they occur opposite bases that have been damaged by oxidation or UVR. Bases can be introduced during recombination if there are differences between the sequences of the DNA strands that form the heteroduplex. Mismatched bases can also occur when imperfect palindromes in the DNA sequence form hairpin structures, or when deamination of a methylated cytosine (5-methylcytosine) in a G:C pair leads to the incorporation of a T opposite the G. In mismatch repair, the mismatched bases do not have to be biochemically altered or damaged; they can simply be in the wrong location. Genes involved in mismatch repair (MMR) are highly conserved between prokaryotes and eukaryotes (for a general review of mismatch repair, see Friedberg *et al*, 1995).

When a mismatched base occurs during DNA synthesis or recombination, it must be recognised as such, and dealt with, so that it does not become a permanent fixture. The correct strand of DNA must be used as a template to resynthesise the base. In

prokaryotes, the distinction between the two DNA strands occurs following replication by determining the methylation state of the newly synthesised strand. As there is a delay between the synthesis and methylation of DNA, mismatches in the daughter strand can be identified after replication as being on the daughter strand, as this strand is undermethylated compared with the parental strand. This ensures the correct strand is repaired, and is referred to as methyl-directed MMR. A number of genes involved with methyl-directed MMR are conserved between prokaryotes and eukaryotes. In *E.coli*, MutS, MutL and MutH are crucial to the MMR process (Rydberg, 1978). Mismatches are initially recognised by the protein MutS (Su *et al*, 1998). MutL is thought to be a secondary recognition factor in MMR due to its ability to bind MutS bound heteroduplex mismatches. MutH distinguishes the two strands on the basis of their methylation state, and has some endonuclease activity that allows it to cut 5' to the mismatch on the daughter strand (Langle-Ronault *et al*, 1987; Lahue *et al*, 1989). The DNA around the mismatch is then unwound by DNA helicase II. The mismatch is excised, and DNA polymerase III synthesises new DNA. It is likely that DNA ligase is required to seal the strands, as the co-substrate for DNA ligase, NAD+ has been shown to be required for MMR. In man, at least six different proteins are involved with MMR. The MSH2 protein forms a heterodimer with either MSH6 or MSH3, for mismatch recognition (Marsischky *et al*, 1996; Kolodner and Marsischky, 1998). MSH6 is required for the correction of single-base mispairs, while both MSH6 and MSH3 may be involved in the correction of insertion-deletion loops. The interaction between the mismatch recognition complex and other proteins necessary for MMR is coordinated by a heterodimer of the proteins MLH1 and PMS2. Other required proteins are thought to include exonuclease I, PCNA, and the DNA polymerases δ and ε (Bertrand *et al*, 1998).

Inherited errors in the MMR system are involved with the hereditary nonpolyposis colon cancer syndrome. Hereditary nonpolyposis colon cancer (HNPCC) is one of the most common cancer syndromes in humans. Germline mutations in one of four major HNPCC-associated MMR genes, MLH1, MSH2, MSH6 and PMS2 are detected in up to 80% of HNPCC families. Defective MMR capacity is associated with other cancers outside the HNPCC syndrome, such as cancer of the prostate (Yen *et al*, 2001), of the bladder (Thykjaer *et al*, 2001) and oesophagus (Uchida *et al*, 2001).

Nucleotide Excision Repair

Nucleotide excision repair (NER) can repair a wide spectrum of DNA damage, including lesions induced by UVR, cross-linking agents, free radicals and interstrand crosslinks (Wood, 1996; Sancar 1996, Lindahl *et al*, 1997). NER is the sole mechanism for the removal of bulky chemical adducts such as CPDs and 6-4PPs induced by UVR.

During NER, a single stranded fragment of around 30 nucleotides (Friedberg, 2003), which contains the lesion, is excised from the double helix. The remaining strand is used as a template to synthesise a new, lesion-free strand. There are 5 stages of NER, a) the recognition of the DNA lesion which is distorting the double helix; b) unwinding of the helix; c) incision upstream and downstream of the lesion (the so-called Dual Incision); d) synthesis of the new DNA strand, and e) ligation of the strand breaks.

Two sub pathways of NER exist, global genome repair (GGR) and transcription-coupled repair (TCR) (Bohr *et al*, 1985; Mellon *et al*, 1987; Mellon and Hanawalt, 1989). GGR acts over the entire genome at a slower rate than TCR, which is more rapid, preferentially repairing expressed genes on the transcribed strand. The main difference between the processes is the way in which the DNA damage is recognised. A complex of the 125-kDa XPC protein and 58-kDa HHR23B recognises DNA damage during GGR, while the stalled RNA polymerase II complex recognises the damage in TCR. Upon recognition of a DNA lesion in GGR the XPC/HHR23B complex binds to the lesion site, distorting the helical structure and leading to partial opening of the helix. The DNA surrounding the lesion is opened further by 3 protein complexes, TFIIH, XPA and replication protein A (RPA). TFIIH is a 9-subunit complex involved in the initiation of RNA polymerase II transcription (Conaway and Conaway, 1989; de Laat *et al*, 1999), NER (Feaver *et al*, 1993, Schaeffer *et al*, 1993; Drapkin *et al*, 1994; van Vuuren *et al*, 1994; Vermeulen *et al*, 1994; Wang *et al*, 1994) and possibly in the regulation of the cell cycle (Feaver *et al*, 1994; Serizana *et al*, 1995). TFIIH is recruited to the damage through its affinity to bind with the C-terminus of XPA. TFIIH contains 2 DNA helicase subunits, XPB and XPD (van Vuuren *et al*, 1994), which are ATP-dependent. XPB and XPD unwind the DNA in opposite directions; XPB is a 3' to 5' helicase while XPD is a 5' to 3' helicase. XPA verifies the damage, and is crucial for the assembly of the rest of the repair machinery.

RPA binds to the undamaged single strand DNA, preventing both the formation of secondary structures and the undamaged single stranded region from being cut, which is important, as it is the template for repair replication. The exonucleases required for the dual incision are the ERCC1/XPF complex at the 5' end, and XPG at the 3' end. Free ERCC1 (33kDa) and XPF (120kDa) are rapidly degraded in the cell (Sijbers *et al*, 1996). They are tightly bound in complex, which is achieved by the interaction of the C terminal amino acids 224 to 227 of ERCC1 and the C terminal amino acids 814 to 905 of XPF. XPG is recruited by the N-terminal domain of XPC, and incises the DNA 6-9 nucleotides (nt) 3' of the lesion (O'Donovan *et al*, 1994; Huang *et al*, 1992). The ERCC1/XPF complex then makes the second incision 15-24 nt 5' to the incision. RPA is responsible for the correct orientation of the endonucleases. When it is bound to the undamaged single strand of DNA, the 3' orientated side of RPA binds the ERCC1/XPF complex, and the 5' orientated side binds XPG (de Laat *et al*, 1998).

The 22-32nt excised fragment leaves a single strand gap, which is still bound by RPA (Mu *et al*, 1996). The single strand gap is filled by DNA polymerase δ or ε and replicating factor C (RPC). Petit and Sancar (1999) showed both polymerases to be proliferating cell nuclear antigen (PCNA)-dependent. PCNA is recruited to the template strand by XPA. RF-C facilitates binding of PCNA onto the template, followed by the DNA polymerase. The DNA polymerase adds nucleotides to the affected strand in a 5' to 3' direction. The last two nucleotides cannot join together, and the repair process is completed with the ligation of the newly synthesised DNA to the original sequence, a reaction which DNA ligase I can catalyse.

Global genome repair and transcription-coupled repair follow the same mechanisms, apart from the initial damage recognition. The XPC/HHR23B complex is not needed in TCR (Venema *et al*, 1990, 1991; van Hoffen *et al*, 1995), as the RNA polII complex that is transcribing the DNA is stalled by the presence of a lesion (de Boer and Hoeijmakers, 2000). Here, the stalled RNA pol II acts as the signal to attract the NER proteins. The rate of repair by GGR varies depending upon the lesion type. For example, 6-4PPs are removed much more quickly than CPDs from the genome, most likely because of differences in the affinity of XPC-HHR23B. The location of a lesion and how accessible it is also affects the lesion removal rate.

Araujo *et al* (2000) demonstrated that the overall genome repair reaction could be reconstituted *in vitro* with recombinant human proteins. Repair can be achieved *in vitro* by the concomitant presence of 10 components, RPA, XPA, XPC/HHR23B, XPG, ERCC1/XPF, TFIIH, DNA pol δ and/or ε, RPC, PCNA and DNA ligase I. However, this is less efficient than NER in whole cell extracts, where accessory proteins like XPE are also involved.

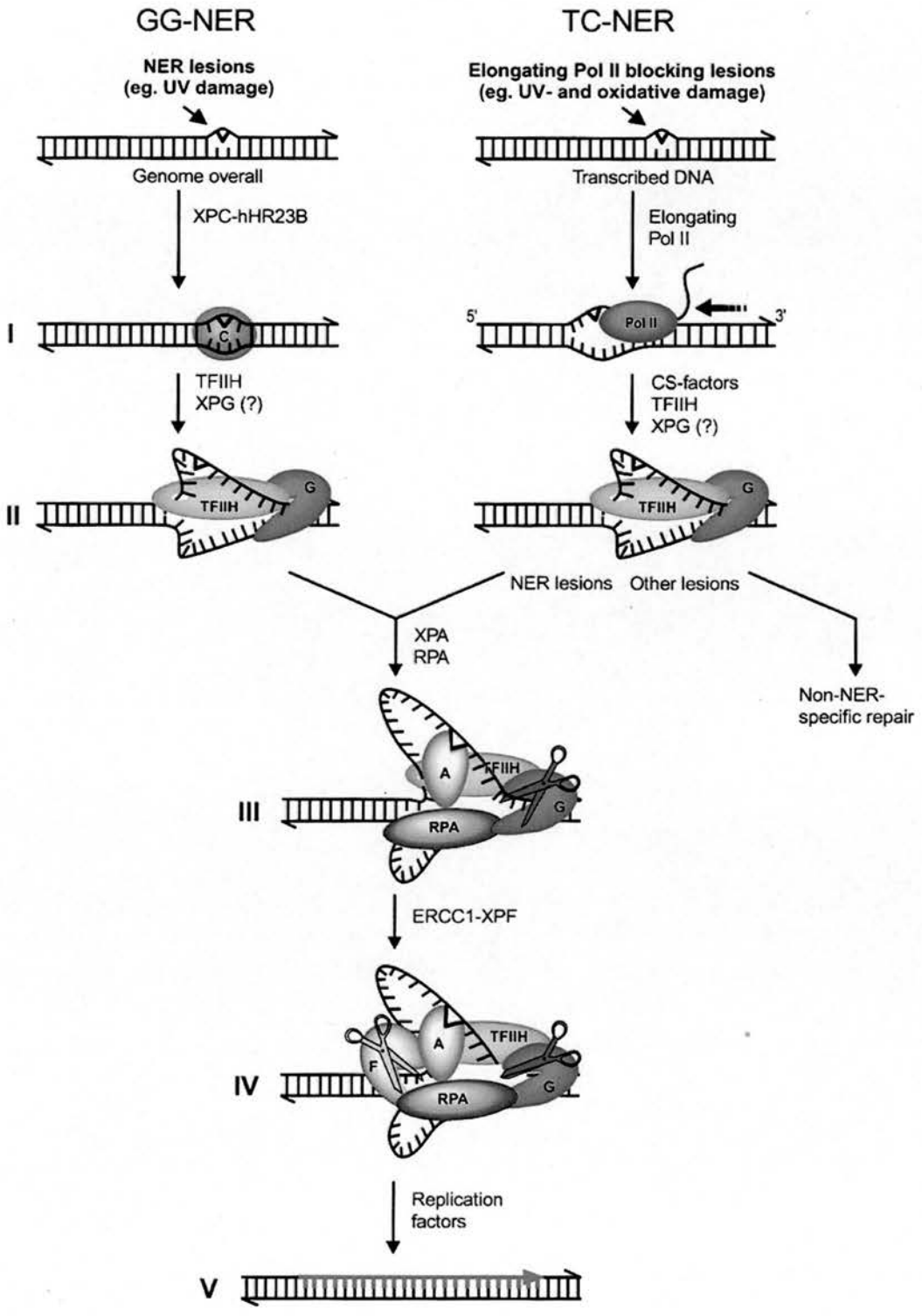


Figure 3, **model for the incision stage of NER. (Adapted from de Laat *et al*, 1999).**

Defects in NER

In contrast to defects in BER, which are embryonic lethal, defects in NER in man can lead to a variety of phenotypes. Defects in NER are associated with three rare recessive syndromes, Xeroderma pigmentosum (XP) (see OMIM entry 278700 and entries therein), Cockayne's Syndrome (CS) (OMIM %216400 and entries therein) and Trichothiodystrophy (TTD) (#601675 and entries therein).

Xeroderma pigmentosum

Xeroderma pigmentosum (Xeroderma meaning parchment skin, and pigmentosum reflecting freckling, both of which are seen in this disease) is a rare, autosomal recessive disorder, where affected individuals are extremely photosensitive. Mutations in several of the genes involved in XP give rise to similar phenotypes (Boulikas, 1996). Its incidence varies according to geographical location, with a frequency of around 1 in 1 million in Europe and the United States, and 1 in 100,000 in Japan (Takebe *et al*, 1987). As a result of the extreme photosensitivity, sufferers have a more than 1000-fold increased risk of skin cancers, both non-melanoma and melanoma on sun-exposed sites, with a mean age of onset of around 8 years of age, 50 years earlier than in the US white population (Kraemer *et al*, 1994). This is one of the largest reductions of age of onset of cancer of any genetic disorder. Stringent measures of avoiding UVR results in a lack of the cutaneous damage usually seen in XP (Davis *et al*, 1994). It is unclear whether the heterozygous genotype, with one wild-type repair gene and one mutated repair gene, has any phenotypic effect, such as increased sensitivity to UVR and a higher skin cancer risk, although increased freckling has been claimed to be such a manifestation. Normal skin fibroblasts can repair UVR-induced DNA damage, however, Cleaver (1968), in a seminal paper, found that fibroblast cells from XP patients have a greatly reduced ability to repair UV-induced DNA damage. Cell fusion experiments identified 7 complementation groups in XP (XP-A to –G), and a variant form, XPV. In XPE and XPV, NER is either normal or only slightly reduced. Defects in XP cell lines from different complementation groups vary in the ability to repair the two main classes of UV-induced mutations, CPDs and 6-4PPs, indicating that different subpathways of NER repair the two types of lesions.

The photosensitivity is due to defective NER, which results in the accumulation of photoproducts that are converted to mutagenic mutations during semi-conservative DNA synthesis. Thompson (1998) determined that the extent of DNA repair deficiency, as measured by cell survival curves and incision activity, correlates with the age at which symptoms develop, and the rate of tumour growth. There is also an increased risk of tumour development on other sites that are exposed to UVR, such as the tip of the tongue and the eyes (Kraemer *et al*, 1987, 1994), as well as an increased incidence of internal tumour development. Patients develop progressive neurological abnormalities in 20% of XP cases, this being more common in XP-A patients than XP-D and XP-G patients. Loss of neurons, particularly in the cerebellum and cerebral cortex (Thompson, 1998) leads to these neurological abnormalities, which can include hyporeflexia, sensorineural deafness mental retardation and microcephaly. There can also be, in some cases, slowed growth and delayed onset of secondary sexual characteristics. The degree of severity of neurological dysfunction also seems to be associated with the level of NER deficiency. Andrews *et al* (1978) demonstrated a correlation between the degree of sensitivity of cultured fibroblasts to UVR (as measured by their ability to form colonies) and the severity of neurological abnormalities. They concluded that DNA repair is required for maintenance of the functional integrity of the nervous system, by preventing the premature death of neurons. Neurological damage is thought to occur due to an inability to repair oxidative DNA damage in the brain.

Swift and Chase (1979) examined the families of 31 XP patients, and observed that significantly more blood relatives had had NMSC than spouse controls. This supports the hypothesis that individuals heterozygous for XP genes may be predisposed to skin cancers, perhaps in association with a substantial amount of exposure to UVR, which could overwhelm their DNA repair genes.

Cockayne's Syndrome

Individuals with CS are also photosensitive, although much less so than those with XP, or those with combined XP/CS, and are not predisposed to skin cancer. Patients with CS suffer a range of symptoms, which include dwarfism, due to arrested growth and

development (despite normal growth hormone levels), progressive neurological degeneration and a shortened lifespan (median life span 12 years). The neurological abnormalities seen in CS are due to demyelination in nerve tissue. Other classical features of the CS phenotype are cataracts, deafness, dental caries, intracranial calcifications, and abnormally large facial features and limbs in proportion to body size.

Cell fusion experiments have identified 5 complementation groups associated with CS, although only those not also associated with XP are formally recognised as CS (CS-A and CS-B). The genes mutated in CS-A and CS-B have been cloned. Orrent *et al* (1996) transfected the normal CSB gene into hamster cells with the CSB phenotype, and found that the TCR-deficiency and UV-sensitivity found in these cells was restored to that seen in normal cells, indicating that the defect in TCR in CSB cells is due to mutations in the CSB gene. Depending on the mutations involved, the CS that is associated with XP is referred to as XP-B/CS, XP-D/CS or XP-G/CS.

XP/CS patients show the same level of photosensitivity as in XP, and will, in some cases, go on to develop skin cancers. They do not, however, develop primary neuronal degeneration, or ichthyosis or brittle hair. There is higher neurodysmyelination than in CS alone, or TTD, but mental retardation and failure to grow is similar to CS and TTD. Cells from patients with XP/CS are more sensitive to UV killing than even XP-A cells, although the repair deficiency is similar to that found in XP-D cells, which are less sensitive to UVR. (Lehman, 2001). Cells from CS patients have UV hypersensitivity due to a defect in NER. UV-induced CPDs (but not other photoproducts) are unable to be repaired from the template strand in actively transcribed DNA as the cells are defective in TCR. RNA synthesis is normally depressed following UVR, recovering after repair of DNA damage. In CS cells, this defect in TCR means that the damage in actively transcribing cells is not repaired and the RNA levels cannot return to normal after DNA damage. A clinical method of diagnosing CS is to measure the recovery rate of RNA synthesis following UV-induced DNA damage. The defect in TCR in CS is not only found after exposure to UVR, but also after exposure to forms of oxidative stress (Cooper *et al*, 1997). This suggests that TCR, and/or the CS proteins, may have additional functions outwith NER.

Trichothiodystrophy

TTD is a term first introduced by Price *et al* in 1980 for sulphur-deficient brittle hair. TTD is an autosomal recessive disorder, comprising a variety of different sulphur-deficient brittle hair disorders, including Pollitt (OMIM %275550), Amish brittle hair brain syndrome (#234050), and Sabinas brittle hair syndrome (OMIM %211390) (Bergmann and Egly, 2001). Approximately half of all TTD patients are photosensitive, which is linked to deficient excision repair as seen in XP cell lines. These TTD patients fall within the definition PIBIDS, standing for Photosensitivity, Ichthyosis, sulphur- and cysteine- deficient Brittle hair, impaired Intelligence (mental retardation, spasticity, tremor and ataxia), Decreased fertility, and Short stature. The severity of symptoms can vary, from mild to extremely severe, leading to a failure to thrive and/or death in early childhood. TTD is not however a cancer prone disease, and patients have no predisposition to skin cancers, as seen in XP (Itin and Pittelkow, 1990; Stary and Sarasin, 1996). Neuronal abnormalities in TTD are predominantly due to demyelination.

Cellular features of the NER deficiency syndromes

Cell fusion experiments have identified 3 complementation groups in TTD; XP-D, which accounts for the majority of TTD patients, XP-B and TTD-A, whose protein is part of the basal transcription factor TFIIH (Thompson, 1998). The most highly photosensitive TTD lines are found within the XP-D complementation group, with the XPD gene being able to correct the repair deficiency in these cells. The ability of XPD to correct the repair defect was explained when it was found to be involved with both NER and transcription.

Only XP is associated with the early onset of skin cancer. All three disorders possess a degree of defectiveness in NER capacity, reflected in the photosensitivity the patients display. Most XP groups are deficient in both GGR and TCR, except XP-C, where only GGR is defective. CS groups are defective in TCR, XP/CS is defective in both. TTD groups are defective in transcription, while the NER level can be normal, intermediate or defective. Transcription-coupled repair, rather than global genome repair is implicated in

the detection and repair of UV induced erythema and resulting DNA damage. Garssen *et al* (2000) examined levels of UVB-induced erythema, as determined as the lowest dose of UV that was able to induce a significant oedema and erythema (the MED), in mouse models. Wild type mice, with efficient GGR and TCR sub pathways of NER had an MED of 1500J/m^2. XPA mice, which are deficient in both GGR and TCR, were significantly more sensitive to UVR, with an MED of <150mJ per cm/m^2. A similar MED was observed in CSB mice, which are efficient at GGR, but deficient in TCR. XPC mice have deficient GGR, but wild type-levels of TCR. XPC mice showed similar MED to wild type mice, i.e. 1500J/m^2. As mice with deficient GGR only had UVR sensitivity equivalent to that observed in wild type mice, and mice which have deficiencies in both GGR and TCR (XPA mice) are no more sensitive to UV than mice with only TCR-deficiency, these results indicate that erythema reactions induced by UV are primarily determined by TCR alone. This data obtained from mouse studies is in agreement with Japanese studies of XP patients. Ichihashi *et al* (1981) and Kondo *et al* (1989) found XPA patients had a low MED, while patients with XPC had MEDs within the range seen in healthy humans (Kondo *et al*, 1992).

The wide clinical heterogeneity seen associated with NER defects is likely due to additional functions of the NER components involved.

Main Clinical Symptoms of the NER syndromes

Clinical Symptoms	XP	XP/CS	CS	TTD
Photosensitivity	++	++	+*	+*
Abnormal pigmentation	++	+	-	-
Skin cancer	++	+	-	-
Progressive mental degeneration	-/+*	+	+	+
Neuronal loss	-/+*	-	-	-
Neurodysmyelination	-	++	+	+
Wizened facies	-	+	+	+
Growth defect	+/-*	+	+	+
Hypogonadism	-/+	+	+	+
Brittle hair and nails	-	-	-	+
Ichthyosis	-	-	-	+

* These XP patients have DeSanctis Cacchione Syndrome, cutaneous XP associated with microcephaly, progressive mental degeneration, dwarfism and immature sexual development.

The identification of XP, CS and TTD groups by complementation studies were mainly based on analysis of repair capacity of the different cell lines. Overlap between XP cell lines and some phenotypically similar rodent cell lines, which contain 11 complementation groups, lead to studies investigating whether human DNA could rescue the rodent phenotypes. A series of genes which, when transfected into rodent mutants, corrected the rodent phenotypes, was cloned, and designated ERCC (standing for Excision Repair Cross Complementing). These are ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6 and ERCC8, and they partially overlap with XP genes (Wood, 1997). ERCC2 is equivalent to XP-D, ERCC3 to XP-B, ERCC4 to XP-F, ERCC5 to XP-G, ERCC6 to CS-B and ERCC8 to CS-A. Mutations in rodent groups 1 and 4 display extreme hypersensitivity to DNA cross-linking agents, such as mitomycin C and cisplatin, a feature which is not seem amongst human NER mutants. Mutant groups 1 and 4 are unique in this respect among the NER-deficient phenotypes in mammalian cell mutations (Thompson, 1998).

DNA Repair Capacity

Variation in DNA repair capacity, as measured by several lymphocyte assays, has been observed in the general population. Individuals with a repair capacity of 65-80% of the population mean have been observed more often in cancer cohorts than in control cohorts in several studies of different cancer types, such as basal cell carcinoma (Wei *et al*, 1994,1995), lung cancer (Wei *et al*, 1996) and squamous cell carcinoma of the head and neck (Cheng *et al*, 1998), although other studies have not observed a reduced repair capacity in patients with basal cell carcinoma (Hall *et al*, 1994) or cutaneous melanoma (Xu *et al*, 2000) suggesting further analysis is required. Repair capacity of 65-80% of the mean has been found, at least by some, to constitute a risk factor for cancer. When this level of repair capacity is compared with that seen in cells from XP patients, which can have a NER repair capacity barely above the experimental background level of around 2% of the general population mean, it is feasible that even small amino acid changes (that

do not lead to XP, TTD or CS) in genes involved with the NER pathway might constitute a cancer risk in the general population. This may also be true of genes involved in other DNA repair pathways.

Genes analysed in this study

XPD (ERCC2)

As previously described, the XPD gene encodes a protein essential to the NER pathway, an 86.9 kDa 5' to 3' DNA helicase which is a component of the TFIIH complex. The TFIIH complex is involved in the initiation of RNA polymerase II transcription and Nucleotide Excision Repair; de Laat *et al* (1999) suggest that it might also be involved with the regulation of the cell cycle. XPD contains 23 exons, which are highly conserved in humans, the hamster (90% identity with human), mouse (89.8% identity) and fish, Xiphophorus maculates (76% identity), (Lamerdin *et al*, 1996), highlighting the evolutionary importance of XPD, although the introns are not conserved. Flejter *et al* (1992) proved that the ERCC2 gene corrected the UVR-sensitivity and defective nucleotide excision repair seen in cells from patients with XP complementation group D, and that ERCC2 and XPD are the same gene. The human XPD maps on the long arm of chromosome 19, bands 13.2-13.3. This same region contains other repair genes, ERCC1 (crucial to the NER pathway) and XRCC1, a component of the BER pathway. It also contains apoliprotein (APO) genes, the muscle creatine kinase (CKM) gene locus, the myotonic dystrophy (DM) gene, and DNA ligase I. Lamerdin *et al* (1996) determined the organisation of this region to be:

centromere....$^{3'}$XRCC1$^{5'}$...APO....$^{3'}$CKM$^{5'}$...$^{3'}$XPD$^{5'}$...$^{3'}$ERCC1$^{5'}$....DM...tel

The CKM, XPD and ERCC1 genes are physically linked within an approximately 250kb segment, therefore if a mutation in the XPD gene gave rise to an increased cancer risk, CKMM and ERCC1 might seem to show an association too.

The XPD protein is 760 amino acids (aa) in length, and it too shows a high degree of homology with its hamster, mouse and fish equivalents. The main structures of the XPD

protein are an ATP binding motif, at aa35-52, a putative nuclear localisation signal (aa234-237) and seven helicase motifs (amino acids 35-51, 69-88, 225-240, 464-478, 539-360, 587-611 and 654-671). Hoeijmakers (1993) and Ellis (1997) identified a DNA binding domain within helicase motif V, one of the regions where the majority of mutations in XP, XP/CS or TTD mutations occur (the others being helicase motifs I, II, III and VI). The N-terminus interacts with the C-terminal domain of p53 (Leveillard *et al*, 1996).

Polymorphisms in the XPD gene in the general population were identified by Shen *et al* (1998), who proposed that they might have associations with increased risk of susceptibility to different cancers.

Vodicka *et al* (2004) studied the frequency of chromosomal aberrations and single strand breaks in DNA in cells from individuals with different genotypes at the exon 23 polymorphism of XPD. Chromosomal aberrations are positively associated with the onset of cancer, particularly in smokers (Bonassi *et al*, 2000). Vodicka *et al* found that the CC genotype had less chromosome aberrations than the AA and AC genotypes (p=0.028), and lower levels of single strand breaks in DNA after gamma irradiation (p=0.033), which might suggest the CC genotype confers some protective affect against the development of cancer, and is more efficient at repairing chromosomal aberrations, or is less prone to them in the first place.

Tomescu *et al* (2001) found associations between 3 polymorphisms in XPD (which were an A to C change in exon 6, a C to T change in exon 22, and an A to C change in exon 23) and an increased risk of cutaneous melanoma. Two of these polymorphisms, in exon 6 and exon 22, do not result in an amino acid substitution, while the third, in exon 23 leads to a Lys→ Gln change. In a study of 28 patients with melanoma and 33 matched-controls the A allele at exon 6 was found to be over- represented in the melanoma cohort, although this was not formally significant (Odds Ratio (OR) 2.0, 95% Confidence Interval (CI) 0.9-4.5, P=0.08. The C allele in the exon 22 polymorphism was also significantly over-represented in the melanoma patients, with an OR 2.6, 95% CI 1.1-6.7 and P value of 0.04. In exon 23, the A allele was more frequent in the melanoma cohort (OR 2.8, 95% CI 1.2-7.0, P= 0.02). Winsey *et al* (2000) investigated the exon 6 and exon

23 polymorphisms in melanoma patients, and found no association between either polymorphism genotype and melanoma risk.

The XPD exon 6 polymorphism has been observed to be associated with an increased risk of basal cell carcinoma (OR 1.9; 95% CI 1.0-3.8, Vogel *et al*, 2001). The association was higher among individuals with no family history of non-melanoma skin cancer. The exon 6 polymorphism has also been studied for possible associations with other cancers. Mort *et al* (2003) examined a cohort of colorectal cancer patients to determine any association between colon cancer and a variety of repair genes. Mismatch repair has been shown to be important for the prevention of the development of colorectal cancer, with deficiencies in MMR seen in 15% of cases. No association was found between exon 6 of XPD and colorectal cancer, or exon 22 or 23, which are also examined here.

The XPD exon 23 polymorphism has been analysed for association with lung cancer by several groups. Carcinogens in tobacco smoke induce lesions in DNA, which are part of the wide range of DNA damage repaired by nucleotide excision repair. As the XPD gene is crucial for efficient NER of DNA, it is possible that if the XPD exon 23 polymorphism is functional it could give rise to an increased risk of lung cancer in smokers. The exon 23 polymorphism was studied in a large, hospital-based study in America by Zhou *et al* (2002) for any association with lung cancer. 1092 cases and 1240 controls were analysed. No increased risk of lung cancer was seen in the overall data set; however, an increased risk of lung cancer was observed in non-smokers (OR 2.0; 95% CI 1.1-3.4). Hou *et al* (2002) found a similar association with smoking status in a smaller study in Sweden, although other investigators have found no suggestion of association or an interaction of the exon 23 polymorphism with smoking status influencing lung cancer risk (Park *et al*, 2002; David-Beabes *et al*, 2001).

The three polymorphisms described in XPD will be analysed to determine any association with increased sensitivity to UVR, as measured by level of UV-induced erythema.

ERCC1

In complex with XPF, ERCC1 makes the 5' incision to damaged DNA during NER. The gene is approximately 15kb in size, and is located close to XPD, on human chromosome 19q13.2. The ERCC1 cDNA gives rise to a transcript of 1.1kb, which encodes a 297 amino acid product. The human ERCC1 gene was used as a probe to isolate the mouse ERCC1 gene. The human and mouse genes are highly conserved, with their proteins showing an 85% amino acid similarity. Human ERCC1 is also homologous to the Saccharomyces cerevisiae RAD10 gene, which links with RAD1 to form an endonuclease, and the Saccharomyces pombe swi10 gene (Rodel *et al*, 1997), highlighting its evolutionary importance.

XPF (ERCC4)

A previously described, XPF and ERCC1 form a complex, which is the endonuclease responsible for making the incision 5' to the damaged DNA during NER. The XPF gene was identified by Sijbers *et al* (1996), who isolated a human gene which was homologous to the yeast gene Rad1, and found it to correct the repair defects seen in cells from patients in the XP group F, as well as the rodent group 4. The XPF gene contains 11 exons, and spans 28.2kb (Brookman *et al*, 1996). As ERCC4, the gene was mapped to chromosome 16 (Siciliano *et al*, 1987), which was refined to 16p13.2-p13.1 by Sijbers and colleagues.

None of the human NER-deficiency disorders, XP, TTD or CS, are associated with mutation of ERCC1, leading to suggestions that ERCC1-deficiency results in an embryonic lethal phenotype. Ercc1-deficient mice were produced by gene targeting by McWhir *et al* (1993). The Ercc1-deficient pups were severely runted, weighing 20% less than their wild-type littermates. The liver cells of these mice were abnormal, resulting in polyploid liver cells, which are normally only found in older mice. The Ercc1-deficient pups died within three weeks of birth, prior to weaning. The human and mouse proteins have an 85% homology, therefore it is possible that a complete lack of ERCC1 function in humans may indeed give rise to an embryonic lethal phenotype. Mutations which give

rise to a less severe genotype could give rise to a reduced repair capacity phenotype in humans, leading to increased cancer risk.

As well as its role in NER, the ERCC1-XPF complex has also been shown to be involved in recombination repair processes distinct from NER. Chipchase and Melton (2002) demonstrated that rodent cells, in which chromosomal aberrations are common after exposure to UVR, which do not express ERCC1 or XPF have a lower ratio of chromatid exchanges to breaks than wild type cells which are not deficient in ERCC1 or XPF. Cells deficient in other genes involved with NER (XPB, XPD and XPG) display similar rations of exchanges to breaks as wild-type cells, implying that ERCC1 and XPF are involved in the formation of UV-induced chromosomal aberrations out with their role in NER. The dual role of ERCC1 and XPF in the defence of DNA against UV-induced damage suggests that polymorphisms of these genes would be good candidates for markers of association with UV-induced erythema, which is itself a marker for UV-induced DNA damage.

XPG (ERCC5)

ERCC5 was localised on chromosome 13 by Siciliano *et al* (1987) and Thompson *et al* (1987) by the study of somatic cell hybrids between normal cells, and Chinese hamster cells which had a repair deficiency. ERCC5's location was further refined to 13q32.3-q33.1 by use of fluorescence in situ hybridisation (Takahashi *et al*, 1992).

The XPG-complementing protein was generated from a 4kb mRNA, which was present in normal amounts in XPG cell lines. An antibody directed against a fragment of the XPG complementing protein inhibited excision repair by normal cell extracts, this activity could be re-established with an XPG/group 5 complementing fraction. ERCC5 and XPG were confirmed as being the same by sequence analysis of the cDNAs by MacInnes *et al* (1993).

The polymorphism at exon 15, a G to C change, which leads to an amino acid change (from Asp to His) has been studied by a number of groups. This change does not result in an XP or XP/CS phenotype.

As mentioned above, XPG patients that have a truncated version of the XPG protein also suffer from CS (Vermeulen *et al*, 1993; Nouspikel *et al*, 1997). In addition to the NER defect, cells from such patients have a reduced ability to remove thymine glycol (Tg), an oxidised DNA base, from their DNA after exposure to ionising radiation (Cooper *et al*, 1997). This defect is most striking in a transcription-coupled repair process, but is also seen in general transcription-independent repair. The main repair pathway for the removal of Tg lesions from DNA is by BER, which is initiated by Nth1. It now appears that in vivo this BER process might be improved by XPG, which has traditionally been thought of as an NER factor. Klungland *et al* (1999) reconstituted BER of oxidative DNA damage using purified human factors and DNA substrates containing toxic or mutagenic oxidised forms of pyrimidines. They found the initial step on the reaction, catalysed by Nth1, to be rate limiting, but that it is strongly stimulated by purified XPG protein, which promotes binding of Nth1 to its DNA substrate, although XPG does not act as a substrate-specific nuclease in this process. This is consistent with cellular studies, which have indicated a function for XPG in the repair of oxidative damage that is distinct from its NER endonuclease activity (Cooper *et al*, 1997; Nouspikel *et al*, 1997).

Vodicka *et al* (2004) found that the exon 15 polymorphism was marginally associated with an increased level of chromosomal aberrations in GG homozygotes as compared with CC homozygotes. Studying the repair of 8-oxyguanines after gamma irradiation in lymphocytes to determine the DNA repair rate, they found the irradiation specific DNA repair capacity to be moderately higher in CC homozygotes compared with the GG homozygotes. This was not significant ($p=0.089$) but is of interest, as it supports the chromosome aberration data in suggesting a protective role of the CC homozygotes.

The exon 15 polymorphism has also been associated with increased risk of lung cancer. Jeon *et al* (2003) studied the polymorphism in 310 lung cancer patients, and 311 matched controls, in Korea, and found the GG genotype to be more frequent in controls than cases, being associated with a decreased risk of lung cancer (OR 0.54, 95% CI 0.37-0.8) compared with the CG and CC genotypes.

XRCC1

XRCC1 (X-ray Repair, Complementing Defective in Chinese Hamster, 1) was identified by its ability to restore DNA repair deficiency in the Chinese hamster ovary cell lines EM-9 and EM-11. These cell lines are hypersensitive to ionising radiation and alkylating reagents (Caldecott *et al*, 1995, Thompson *et al*, 1990; Zdzienicka *et al*, 1992). These cells have increased rates of spontaneous and mutagen-induced sister chromatid exchanges, and have defects in rejoining single-strand breaks after exposure to X-rays (Thompson *et al*, 1982; Zdzienicka *et al*, 1992). Both EM-9 and EM-11 cells have a mutated XRCC1 gene, and lack XRCC1 protein. The human XRCC1 gene consists of 17 exons, spanning 31,9kb (Lamerdin *et al*, 1995).

Shen *et al* (1998) identified three coding polymorphisms in XRCC1 at codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln), of which the polymorphism at codon 399, in exon 10, is the most extensively studied. Poly (ADP-ribose) polymerase (PARP) detects DNA strand breaks induced by ionising radiation, and is believed to participate in BER. XRCC1 negatively regulates PARP by binding to it via the XRCC1 central domain. The exon 10 polymorphism is within this PARP binding domain, therefore any functional effects of the polymorphisms could ultimately affect the repair of DNA damage normally repaired by BER. Various studies have investigated whether the exon 10 R399Q (a G to A base change) polymorphism confers an increased risk of different types of cancer. These studies have suggested associations with risk of cancer in different directions for different cancer types. Nelson *et al* (2002) reported the AA genotype lead to a decreased risk of non-melanoma skin cancer, for basal cell carcinoma OR=0.7, 95% CI 0.4-1.0 and for squamous cell carcinoma OR=0.6, 95% CI, 0.3-0.9. This genotype was also seen to confer a decreased risk of oesophageal cancer (Lee *et al*, 2001) amongst drinkers (OR= 0.4; 95% CI 0.1-0.9) and of bladder cancer (Matullo *et al*, 2001; Stern *et al*, 2001). Duell *et al* (2001) studied the association of the exon 10 polymorphism, and found it to confer an increased risk of breast cancer in African Americans (OR 1.7; 95% CI 1.1-2.4). Studies of the effect of the polymorphism on squamous cell carcinoma of the head and neck (SCCHN) gave inconsistent results. Sturgis *et al* (1999) observed an increase in risk associated with the AA genotype, (OR, 1.6; 95% CI, 1.0-2.8) while Olshan *et al* (2002)

observed a decrease in risk (or, 0.1; 95% CI 0.04-0.6). No association was seen between this polymorphism and melanoma (Winsey *et al*, 2000).

XRCC3

The human XRCC3 gene is located on chromosome 14, position 14q32.3 (Tebbs *et al*, 1995). The XRCC3 gene was shown to correct the X-ray cross-linking sensitivities and spontaneous chromosomal aberrations of the CHO cell line irs1SF (Tebbs *et al*, 1995). This cell line is hypersensitive to ionising radiation, and cross-sensitive to a range of DNA-damaging agents, including UVR, ethyl methanesulphonate, mitomycin C and cisplatin.

XRCC3 is involved with the homologous pathway of repair of double stranded DNA, preventing the chromosomal fragmentation and the accumulation of translocations and deletions. XRCC3 is structurally similar to Rad1, a component essential for recombination repair. Cells deficient in XRCC3 fail to form Rad521 foci following exposure to radiation, and display genetic instability and an increased sensitivity to DNA mutagens, such as UVR. It is therefore possible that subtle mutations in the XRCC3 gene could affect sensitivity to UVR in humans.

In a study of the association between polymorphisms in DNA repair genes and the development of malignant melanoma, a polymorphism in exon 7 of XRCC3 was investigated (Winsey *et al*, 2000). The C to T polymorphism at position 18067 was genotyped in 125 individuals with malignant melanoma. The presence of a T allele was seen to be significantly associated with melanoma development (p=0.004, OR 2.36). As UVR is the key causative agent for development of malignant melanoma, it is plausible that polymorphisms associated with melanoma will also associate with UVR-induced erythema.

p53

Mutations and deletions of the p53 gene (OMIM +191170) that lead to a loss of tumour suppressor function and cell cycle control are found in more than 50% of all human cancers, in more than 50 different types of cells and tissues. Single base pair substitutions

comprise the most common mutational events, followed by single base frameshifts and small deletions. In addition to germline mutations, mutations develop due to exogenous mutagens or endogenous mutagenic processes which damage the genome in characteristic ways, leaving so-called "mutagen fingerprints" in the DNA (Greenblatt *et al*, 1994). Examples of mutation fingerprints in p53 are the G: C to T:A transversion in codon 249, which Hsu *et al* (1991) observed at high frequencies in hepatocellular carcinomas in regions where there was a high ingestion of the mutagen aflotoxin, and the characteristic mutations produced by UVR. UVR induces a high fraction of CC to TT tandem mutations, or C to T transitions at dipyrimidine sites.

Loss of p53 activity is strongly selected for in tumour development. p53 can be inactivated in several ways, including mutation, the loss of part of chromosome 17p, which harbours the gene, or the binding of products of certain oncogenic tumour viruses to the wild-type protein, for instance the large T antigen of SV40, which led to the original identification of p53. Binding of the human papilloma virus 16 E6 protein to wild-type p53 results in a loss of function of the p53 protein, and along with other transforming genes for HPV appears to contribute to the ability of HPV to transform cells, at least in vitro.

Li-Fraumeni syndrome (OMIM # 151623) highlights the role of p53 as a tumour suppressor. Li-Fraumeni syndrome is a rare, autosomal dominant syndrome, where affected individuals carry heterozygous mutations of p53 in the germline. DNA damage leads to the acquisition of further mutations, and the loss of the wild-type p53 allele. Consequently, affected individuals develop a variety of mesenchymal and epithelial tumours at an early age, including breast cancer, soft tissue sarcomas, brain tumours, osteosarcoma, leukaemia and adrenocortical carcinoma (Li and Fraumeni, 1969, 1982; Birch *et al*, 1984, 1990).

The p53 protein is normally present at low levels in the cell, but can be stabilised, by, for example, DNA damage or hypoxia. The E3 ubiquitin ligase, MDM2, closely regulates p53 under normal circumstances, mediating both the ubiquiylation and proteasome-dependent degradation of p53 (Michael and Oren, 2003). p53 and MDM2 function within an auto regulatory loop. The mdm2 gene is itself a p53 target, and p53 positively

regulates MDM2 expression, while MDM2 negatively regulates p53 levels and activity (Wu *et al*, 1993). DNA damage shown to activate p53 includes that generated by UVR, ionising radiation and radiomimetic drugs. The biological consequence of p53 upregulation is the initiation of processes that lead to either cell cycle arrest or apoptosis. After DNA damage has occurred, the p53 protein is accumulated in cells, then translocates to the nucleus and activates gene transcription. p53 acts as a transcription factor, binding to specific sites in the regulatory regions of p53-responsive genes. Different stresses can act through different processes to interrupt the p53-MDM2 regulatory cycle, but all lead to the induction of the p53 protein and its biological consequences. UVR and DNA strand breaks inhibit the ability of MDM2 to mediate p53 degradation, while hypoxia is thought to act on the regulatory loop by repressing the expression of mdm2. Hyperproliferation, induced by oncogenes, can induce the expression of p14ARF, which binds to MDM2, blocking its ability to mediate ubiquitylation and degradation of p53. This results in either cell cycle arrest, or programmed cell death (apoptosis). By initiating cell cycles arrest, there is time for damaged DNA to be repaired before new DNA synthesis occurs, thus preventing the accumulation of mutations in the genome. Apoptosis in cells with excessive damage, where the damaged DNA cannot be repaired has a similar outcome; permanently removing damaged and mutated cells from the population of dividing cells, ensuring any mutations cannot be replicated.

Nucleotide excision repair (NER) can eliminate a variety of UV-induced lesions, such as cyclopyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs) from the genome of UV-exposed cells. NER includes two distinct pathways, global genome repair (GGR), which removes lesions from the entire pathway, and transcription coupled repair (TCR) which repairs DNA damage in the transcribed strand of actively transcribing genes. Ford and Hanawalt (1995, 1997) have shown that p53 is required for the efficient nucleotide excision repair through the GGR pathway of UV-induced cyclopyrimidine dimers from genomic DNA. Adimoolam and Ford (2002) then went on to investigate whether p53 regulates the expression of genes required for GGR, and found the NER gene XPC to be DNA-damage inducible and regulated by p53. It is therefore plausible to ask whether a polymorphism of the p53 gene could affect sensitivity to UVR.

The polymorphism at codon 72, in exon 4, of p53 was first determined by Harris *et al* (1986). A single base change occurs, with either a G or C nucleotide present in the middle position of the codon. This leads to a nonconservative amino acid change, resulting in either an arginine, (CGC), or a proline, (CCC), at codon 72. It had been shown that a number of human transformed cell lines expressed more than one discrete p53 protein, as did human primary tumours, although analysis of human genomic DNA with p53 cDNAs revealed a single p53 gene. The two different p53 protein species expressed in human transformed cell lines are distinguishable by their different rates of migration during SDS-polyacrylamide gel electrophoresis.

In investigating how the single p53 gene could code of more than one p53 protein Harris *et al* detailed the codon 72 polymorphism. Using a cDNA library derived from the SV-80 cell line, which expresses in vivo two discrete forms of p53 protein, individual cDNA clones, which encoded one of the two p53 proteins, were isolated. Sequence analysis of these revealed a single base pair difference between the different cDNAs. Two clones, of the same size and restriction map, which coded for different proteins showed the single base change. One clone had a guanine, while the other contained a cytidine, resulting in arginine being expressed by the first clone, and a proline by the later. The faster migrating species expressed an arginine, which has a large side chain, and is positively charged in physiological conditions. In contrast, proline, the equivalent residue expressed by the slower migrating protein, has a small, nonpolar side chain. Similar studies by Matlashewski *et al* (1987), which found both arginine and proline at codon 72 of p53 in clones from SV80 cell lines, lead to this change becoming a well documented polymorphism.

Thomas and colleagues (1999) suggested that the polymorphic variants of codon 72 might have distinct biological and chemical properties. The p53 Arg protein was found to be more susceptible to degradation induced by the HPV E6 protein than p53Pro, which suggested that individuals homozygous for the Arg allele might have an increased susceptibility to HPV-induced tumours. Many different molecular epidemiological studies have investigated the association between the codon 72 polymorphism and the risk of cancer. These include cancer of the bladder (Soulitzis *et al*, 2002), cervix (Helland *et al*, 1998; Josefsson *et al*, 1998; Andersson *et al*, 2001; Zehbe *et al*, 2001; Saranath *et*

al, 2002), head and neck (Sourvinos *et al*, 2001; Shen *et al*, 2002), lung (Fan *et al*, 2000; Wu *et al*, 2002), non-melanoma skin (Marin *et al*, 2000;Bastiaens *et al*, 2000, Shen *et al*, 2003) and prostate (Henner *et al*, 2001). However, much controversy still surrounds whether any of these studies have conclusively linked any of the p53 codon 72 genotypes with risk of malignancy.

The allele frequency of the p53 codon 72 polymorphism has been shown to vary in different populations. Beckman *et al* (1994) investigated allele frequencies in individuals from different ethnic groups, and found a statistically significant correlation between the frequency of the Pro allele and latitude. They suggested that alleles that encode a proline at position 72 might be advantageous in environments subject to high levels of UVR.

McGregor and colleagues (2002) report an association between the allelic variants at codon 72 and sunburn and non-melanoma skin cancer, with Pro/Pro homozygotes having a higher minimal erythemal dose (MED) i.e. less sensitive to UVR. Shen *et al* (2003) have also reported an association between these variants and melanoma, with the Pro homozygotes being less at risk. They analysed 289 melanoma patients, and 308 cancer-free controls, all of who were non-Hispanic whites, finding an Arg allele frequency of 78.2% in cases, and 73.2% in controls, with a p value of 0.045. Genotype frequencies of Arg/Arg, Arg/Pro and Pro/Pro were 62.6%, 31.1% and 6.2%, respectively, in the cases, and 53.9%, 38.6%, and 7.5% respectively in the controls (p=0.096). Using logistic regression analysis they found that the Arg/Arg genotype was associated with an increased risk of melanoma (odds ratio = 1.43, 95% confidence interval = 1.02-2.02) compared with other genotypes, particularly in the over 50s (Odds Ratio (OR)=2.32, 95% CI= 1.39-388). However, these findings are not conclusive in proving a link between the Arg allele and increased risk of cutaneous melanoma, as the difference in genotype frequencies between cases and controls not being formally significant. An association between the allelic variants of the p53 gene and UVR sensitivity is important, as it may provide insight into the mechanics of the reported association with skin cancer.

Not all studies have however shown an association between the p53 codon 72 polymorphism and cutaneous malignancies. Bastiaens *et al* (2001) genotyped the codon 72 allele in 86 subjects with squamous cell carcinoma, 168 healthy controls, 121 with

basal cell carcinoma and 108 subjects with non-familial malignant melanoma. They found no association between any of the alleles and any of the cutaneous malignancies.

In their investigation of the relationship between p53 codon 72 polymorphisms and susceptibility to sunburn and skin cancer, McGregor and colleagues determined susceptibility to sunburn as the minimal dose of solar-stimulated radiation required to induce just perceptible radiation on previously unexposed buttock skin.

The same p53 variants will be examined in this thesis with relation to UVR sensitivity, but will be measured not by the MED as in the McGregor study, but by use of reflectance spectrophotometry. The MED is a threshold measurement, and can be influenced by basal pigmentation. Reflectance spectrophotometry with examination of a range of UV doses at two different body sites will give a more reliable indicator of any association between the allelic variants at codon 72 and sensitivity to UVR.

GSTT1

The GST genes encode enzymes which catalyse the conjugation of glutathione (GSH) and are involved in the detoxification of a variety of reactive and mutagenic compounds, including the products of oxidative stress. GSTs are thought to have evolved to protect cells against reactive oxygen metabolites, and the proteins are found in all prokaryotic and eukaryotic systems, where they can be seen in the cytoplasm, microsomes and mitochondria (Laughlin *et al*, 1998). Oxidative stress can be caused by a variety of cellular processes, environmental agents and chemicals, including UVR and anthralin. The crucial role of GST genes in the protection of cells from cytotoxic and mutagenic effects suggests that polymorphisms associated with a reduced enzyme activity will give rise to an increased susceptibility to a wide range of diseases.

At least seven separate classes of soluble GSTs have been identified to date; these are alpha, mu, pi, sigma, theta, kappa and zeta. The enzymes were classified on the basis of substrate specificity, structure, amino acid sequence and behaviour of the enzyme.

The theta class of GSTs was discovered by Meyer *et al* (1991), and have different catalytic activity compared with other GSTs (Jemth *et al*, 1997). The theta class of GSTs have a Ser-11 residue, which replaces the tyrosine found in the N-terminal of the alpha,

mu and pi classes. Board *et al* (1995), Blocki *et al* (1993) and Liu *et al* (1992) determined that the residue in this position has a key role glutathione deprotonation and activation. This residue also accounts for the particular enzymatic behaviour of the theta GSTs (Meyer *et al*, 1991, 1993). Mutation of Ser-11 inactivates the theta enzyme by raising the pKa of bound GSH, and lowering the turnover number (Board *et al*, 1995; Carruri *et al*, 1997). The serine residue, as opposed to the tyrosine, can form a hydrogen bond with the glutathionyl sulphur atom; this can be responsible for the differences in affinity towards GSH in the theta class compared with the other GST classes.

Deletion polymorphisms in GSTT1 are common in the Caucasian population, with a frequency of around 20%. This null genotype results in a lack of functional enzyme activity. The rate of enzymatic activity has been shown to be around ten-fold higher in the theta class compared with the alpha, mu and phi classes, and affinity towards the glutathione-conjugates is much lower in GST thetas than in other classes, therefore some conjugate products can inhibit the alpha, mu and phi classes of GSTs much easier than they can the theta class (Meyer *et al*, 1993; Ouwerkerk-Mahadev and Mulder, 1998). The biological importance of the theta class is suggested at due to its remarkable conservation in all organisms studied. Theta-like GSTs have been shown to be present in mammals such as man, mouse and rat, but also in shellfish (Hiltonen *et al*, 1996), a variety of insects including the Australian sheep blowfly (Carruri *et al*, 1997; Board *et al*, 1994), the insect Orthosia gothica (Egaas *et al*, 1995), maize (Gronwald *et al*, 1998) and bacteria (Laughlin *et al*, 1998; Pemble and Taylor, 1992).

The human GSTT1 and GSTT2 genes are separated in the genome by approximately 50kb, and are of similar structure, each with five exons with identical introns/exon boundaries (Coggan *et al*, 1998). The GSTT1 is 8.1kb long, while the GSTT2 gene is 3.7kb. A phenotype in humans where glutathione-conjugation of halomethanes, which result in DNA adducts, has been described, with conjugator and non-conjugator phenotypes (Hallier *et al*, 1993). This polymorphic phenotype is due to a deletion of part of the GSTT1 gene, determined by Pemble and colleagues in 1994. This deletion results in no functional enzyme being synthesised. The GSTT1 null genotype varies in

distribution worldwide. Chinese and Korean populations have among the highest frequencies of the null genotype observed, Nelson *et al* (1995) found the null genotype frequency to be 64.4% in Chinese and 60.2% in Koreans. The null genotype is found in around 20% of Caucasians (Brockmoller *et al*, 1996), with lower prevalence in Mexicans (9.7%, Nelson *et al*, 1995) and Finns (13.3%, Saarikoski *et al*, 1998). The null genotype has been suggested to lead to increased risk of a variety of cancers, due to its inability to detoxify endogenous or exogenous carcinogens. As the GSTT1 null genotype results in no functional protein being produced, an individual with such a genotype has a reduced ability to conjugate GSH with a variety of DNA damaging agents, for example, dibromomethane, dichloromethane, ethylene oxide and methyl bromide (Pemble *et al*, 1994; Guengerich *et al*, 1995; Sherratt *et al*, 1997;). Cells in individuals with the GSTT1 null genotype are as a result subjected to greater levels of DNA damaging agents than in individuals who produce the functional GSTT1 protein, who are better able to withstand harmful substances due to their detoxification ability. Reduced ability to deal with DNA damaging agents means a greater amount of DNA damage will occur, increasing the risk of accumulation of deleterious mutations and the possibility of cancer development. Smoking related cancers have been among the most frequent cancer types studied for association with the GSTT1 null allele. In a study of laryngeal carcinoma patients, Jahnke *et al* (1996) found the frequency of the null allele to be slightly increased in patients compared with controls (p=0.064, OR 0,5, 95% CI 0.24-1.05). Oude Ouphis *et al* (1998) examined 185 patients with SCC of the head and neck (SCCHN), 78 patients with benign head and neck lesions, and 201 healthy controls. They found no difference in the GSTT1 null allele frequency between the SCCHN patients and controls, but found the null allele to have a higher frequency in patients with benign lesions of the head and neck compared with the controls (33.3% compared with 20.3%, p=0.03). In a different study of 42 patients with SCCHN and 42 matched controls, Trizna *et al* (1995) found the absence of the GSTT1 allele to give rise to an increased risk of this cancer (OR 2.18, CI 0.91-5.23). From these studies, there is no conclusive evidence to suggest the presence or absence of the gene gives rise to an increased risk of these cancer types. It is of note that almost all studies involving the GSTT1 null polymorphism only detect either the presence or

absence of the gene, and do not differentiate between homozygous and heterozygous status.

As UVB exposure results in oxidative stress, and many of the products of oxidative stress are detoxified by GSTT1, the null allele has been suggested to be a risk factor for sensitivity to UV, and thus susceptibility to cutaneous carcinogenesis. Kerb *et al* (2002) investigated the effect of the GSTT1 polymorphism on the response to UVB, as measured by the MED. 110 healthy Caucasians were genotyped for the GSTT1 polymorphism, using a PCR reaction that allowed the heterozygous and homozygous genotypes to be differentiated. They reported that subjects homozygous for the GSTT1 null allele had lower MED than those who were heterozygous, or homozygous for the functional allele ($p<0.001$). Kerb and colleagues suggested that this suggested the absence of the functional GSTT1 allele gives rise to increased sensitivity to UV. Concurrently to this paper being published, GSTT1 was considered a candidate gene for sensitivity to UV in this study. As previously mentioned, the MED is not an ideal measure of sensitivity to UV, being open to error. The use of reflectance spectrophotometry to determine levels of erythema via an erythema meter should provide an increased accuracy in order to investigate this polymorphism further.

Selection of study groups

Two groups of subjects were used for this study. The first group consisted of 74 healthy volunteers, with no history of skin disease. Group 2 (n=31) were patients undertaking MED phototesting before phototherapy for the treatment of psoriasis. Patients were under the care of Prof. JL Rees. All studies had appropriate ethics committee approval. All volunteers gave informed, signed, consent for the study in which they participated. None of the volunteers received fees for taking part in any experiment.

Group 1, the larger of the two study groups, was composed from volunteers, who were recruited via the local media from the local population. Volunteers had no history of skin disease and were not selected for on the basis of skin type or any other factor which could influence sensitivity to UVR. There was no evidence to suggest this study group did not represent a random sample of the local population. Group 2 consisted of patients attending the Dermatology Outpatients department to receive treatment for psoriasis, by phototherapy. All members of the second group therefore had active skin disease at the time of phototesting. As phototherapy is less efficient in individuals with darker skin types, it is likely that the second group contained a lower proportion of individuals with darker skin types than by chance. It is possible that the presence of active skin disease in these patients could have had some unrecognised effect on their cutaneous sensitivity to UVR. However, as all patients in group 2 had active psoriasis and were being compared with each other and not directly against the volunteers from group 1, this should not affect the analysis of variation within each group. In neither group was there any obvious selection for level of sensitivity to UVR.

Phototesting

Group 1 were irradiated on the lower back using a range of UVB doses (119-300mJ/cm^2) from a lamp unit designed by Prof. Brian Diffey, (Regional Medical Physics, Newcastle, UK), containing a Philips 9w/12 tube. Volunteers in group 1 were phototested by Dr Lisa Naysmith and Ms Karen Waterston, Department of Dermatology.

In Group 2, patients were irradiated on the inner forearm with a similar luminaire with six doses of UVB from a TLO1 Philips tube (0.47-1.5J/cm^2).

Erythema was measured at 48 hours in group 1 and at 24 hours in group 2, using a reflectance instrument (Dia-Stron, Andover, UK), with readings taken in triplicate. (Erythema measured at 24 hours in group 2 to minimise disruption to patients' routine care and at 48 hours in Group 1 due to time restrictions). Baseline reflectance measurements were also taken in triplicate of adjacent unirradiated skin. UVR-induced erythema was defined as the increase in reflectance from the baseline values.

UVR sources

Group 1 were phototested with a broadband UVB source, described above, and designed by Prof. Brian Diffey with ($\sqrt[3]{2}$ increments). A TLO1 UV unit was used to phototest the volunteers in group 2. TLO1 is a narrowband UVB UV source, used in phototherapy. TLO1 lamps emit an approximately monochromatic spectrum, at a wavelength of 311nm.

Erythema Meter

The reflectance instrument obtained from Dia-Stron is based on the work by Diffey and Farr (1984), and utilised reflectance spectrophotometry. Haemoglobin present in the blood vessels in the upper dermis is the main cutaneous chromophore of green light. Cutaneous erythema can be quantified by illuminating the skin with white light, and measuring the levels of green and red light re-emitted from the skin according to the equation:

Erythema index = $\log_{10}$ (Intensity of red component of reflected light/ Intensity of green component of reflected light).

The erythema meter used here contains a tungsten-halogen lamp, which shines white light into a fibre optic probe. The probe is held against the skin, illuminating it, and then collects diffuse light scattered from within the skin. The instrument contains narrow band interference filters, which the reflected light passes through. The filters are at three wavelengths, of which two, at 546nm (green) and 632nm (orange/red) are used to

determine erythema. The erythema index determined by Diffey and Farr is multiplied by 1000, and the instrument has an output of +999 to –999 erythema units.

Anthralin Exposure

Doubling doses of anthralin (also known as dithranol) (0.01%, 0.02%, 0.04% and 0.08% in final volume of 0.15mL) in Lassar's paste (24% zinc oxide, 2% salicylic acid, 24% starch, in white soft paraffin, 50%) were applied to the mid-back. Lassar's paste alone was applied as a control. All applications were occluded under 8mm Finn chambers (Epitest Ltd, Hyryla, Finland) and secured with Mepore tape (Tendra, Mölnlycke Health Centre AB, Göteborg, Sweden). Applications were left *in situ* for 24 hours. The resulting erythema at all sites was measured at 48 hours, by Contact laser Doppler flowmetry (moorLAB satellite, Moor Instruments Ltd., Axeminster, UK). Laser Doppler flowmetry was used in order to circumvent the potential for confounding by pigmentation. Baseline values were taken an adjacent skin. Doppler flux is expressed as an arbitrary number in "flux units" (Farr and Diffey, 1986). The resulting erythema was defined as the increase in flux units after subtraction of baseline values.

MOLECULAR BIOLOGY

Unless otherwise stated, all common molecular biology reagents were obtained from Sigma.

Collection of Blood for DNA analysis

Blood samples were obtained from each participant in this study for the purpose of DNA analysis. 20mls venous blood was collected in Heparin tubes, and 10ml stored at –20˚C for a period of no longer than one month before DNA extraction. 10mls from each volunteer were kept in the original tube at –70˚C for long term storage.

Extraction of DNA from Blood

DNA was extracted from blood using the BACC3 DNA extraction kit from Nucleon. The principle steps involved in the BACC3 protocol are cell lysis by Reagent A (see below for details), deproteinisation with sodium perchlorate, followed by DNA extraction with

chloroform and the Nucleon resin. The Nucleon resin covalently binds proteins and forms a semi-solid stratum during partitioning. This traps the protein-containing material at the interface and in the organic phase after centrifugation. The DNA-containing upper phase can then be easily removed and the DNA washed with ethanol.

The collected blood was mixed with 4 times the volume Reagent A, and mixed for 4 minutes at room temperature. It was then centrifuged at 1300g for 5 minutes. The supernatant was discarded without disturbing the pellet. The pellet was re-suspended in 2ml Reagent B. 500µl of sodium perchlorate solution was added, and the sample mixed by inverting at least 7 times. 2ml of chloroform were then added, and mixed as above, before the addition of 300µl of Nucleon resin. Samples were centrifuged at 1300g for 3 minutes, after which the upper phase was transferred to a clean tube. 4 volumes of cold absolute ethanol were added, and the tube inverted until the precipitate appeared. DNA was pelleted by centrifugation at 4000g for 5 minutes, and the supernatant discarded. The DNA was washed with 2ml cold 70% (v/v) ethanol, and re-centrifuged. The supernatant was again discarded, and the pellet allowed to air dry for 10 minutes ensuring the removal of all the ethanol. The DNA was then re-dissolved in an appropriate volume of ddH_20 (e.g. 1.0 –2.0ml).

Reagent A
10mM Tris-HCl
0.32M sucrose
5mM $MgCl_2$
1% (v/v) Triton-X-100
Adjust to pH 8.0 using 40% (w/v) NaOH.
Autoclave at 121°C for 15 minutes.

Phenol/Chloroform Extraction of DNA

1 µl of each newly extracted DNA sample was run on an agarose gel to check DNA quality. Samples which had a carry over of other cell material were treated to purify the DNA.

400µl of each DNA sample was used; to which 400µl PCA was added. The samples were placed on a shaker for 15 minutes at room temperature, centrifuged at 13000rpm for 5 minutes and the resulting upper layer transferred to a fresh tube. This process was repeated. 500µl of CA was then added, the sample placed on a shaker for 15 minutes as

above, then centrifuged at 13000rpm for 5 minutes and the upper layer transferred to a fresh tube. 500µl propan-2-ol was added, and the sample inverted until precipitate formed. The sample was left to stand for 10 minutes, before centrifugation at 13000rpm for 3 minutes. The pellet was rinsed in 1ml 70% ethanol, and centrifuged at 13000rpm for 3 minutes. The supernatant was discarded, and the pellet allowed to air dry for approximately 1 hour, before redissolving it in 50-200µl dH_20 (depending on pellet size).

Phenol, Chloroform, iso-amyl alcohol solution

24ml chloroform
1ml iso-amyl alcohol
25ml phenol

Chloroform, iso-amyl alcohol solution

48ml chloroform
2ml iso-amyl alcohol

Polymerase Chain Reaction (PCR)

PCR was carried out using Promega Taq, with a PCR mix made up as below. Approximately 100ng template DNA was used, and a negative control without DNA was run for each reaction. Oligonucleotides were supplied by Sigma-Genosys. Reactions were run on either a Techne PHC-2 PCR machine, in which case each sample was overlaid with ~2 drops of mineral oil to stop evaporation, or a Biometra T3 Thermocycler machine, which has a heated lid to prevent evaporation, eliminating the need for mineral oil. If evaporation did occur, the concentration of the reagents in the reaction mixture would be altered, which could lead to no amplification occurring, or a reduction in the amount of amplification.

PCR master mix

Forward primer	0.5µl
Reverse primer	0.5µl
PCR buffer	5µl
EDTA	2µl
dNTPs	1µl
Promega Taq	0.5µl
ddH_20	37µl

10 x PCR buffer
50mM KCl
1.5mM $MgCl_2$
10mM Tris-HCl, pH 8.3
0.01% w/v gelatin
0.45% v/v Triton X-100
0.45% v/v Tween 20

PCR products were detected by agarose gel electrophoresis. 10µl of each sample were mixed with 3µl sample buffer, and loaded onto an agarose gel.

PCR Primers

Gene/Exon	Primer sequence	product (bp)	PCR Conditions
XPD exon 6	(f) TGTCCAAAACCCCAGCCAGCTG (r) CAGGGGTCAGGGAGGCTGCCTG	288	30 cycles: 94°C 1min, 69°C 1min, 72°C 30s
XPD exon 22	(f) AATGACCTTCTGTCCCTGGCCTGCG (r) AGAAGCTCAGCCTGGGAGGGTGCCG	229	35 cycles: 94°C 1min, 72°C 30s
XPD exon 23	(f) TCAAACATCCTGTCCCTACTGGCCAT (r)CTGCGATTAAAGGCTGTGGACGTGAC	344	35 cycles: 94°C 1min, 67°C 1min, 72°C 30s.
ERCC1 exon 4	(f) TCATCCCTATTGATGGCTTCTGCCC (r) GACCATGCCCAGAGGCTTCTCATAG	252	35 cycles: 94°C 1min, 69°C 1min, 72°C 30s.
XPG exon 15	(f) GACCTGCCTCTCAGAATCATC (r) CCTCGCACGTCTTAGTTTCC	271	35 cycles: 94°C 1min, 62°C 1min, 72°C 1min.
XPF exon 11	(f) TCTCCATGTCCCGCTACTAC (r) GCAGGCACAGGCAAGTTCAA	709	35 cycles: 94°C 1min, 67°C 1min, 72°C 1 min.
XRCC1 exon 10	(f)CCCAAGTACAGCCAGGTCCTAG (r)AGTCTGACTCCCCTCCAGATTC	154	35 cycles: 94°C 30s 58°C 30s

			72° C 30s
XRCC3 exon 7	(f) GCTCGCCTGGTGGTCATCGACTC (r) CTGTCACCTGGAAGAGCACAGTCC	346	35cycles: 94°C 1min, 69°C 1min, 72°C 1min.
CKM 8	(f) TGATCGGCTGGGCTCGTCCGAAGTAG (r)CAGCTTGGTCAAAGACATTGAGGTGG	1099	35 cycles: 94°C 1 min, 67°C 1 min 72°C 1 min
p53 codon 72	(f) ATCTACCGTCCCCCTTGCCG (r) GCAACTGACCGTGCAAGTCA	296	94°C 4 min 35 cycles: 94°C 40s 56°C 30s 72°C 30s 72°C 10 min
GSTT1 wild-type	(f)CCAGCTCACCGGATCATGGCCAGCAC C (r) GGCCTTCCTTACTGGTCCTCACATCTCC	466	95°C 1 min then 35 cycles: 94°C 1 min 65°C 1 min 72°C 1 min. 72°C 10 min
GSTT1 null	(f) TCACTGCCAACTCCAACTCCTGGGTTC (r) TCCGAAGCAACTCCAACTCCTGGGTTC	1498	95°C 1 min, 35 cycles: 94°C 1 min 68°C 1 min 72°C 2 min. 72°C 10 min
rs4150265	(f)GCAGTATGTGAATAGGGGTACAAGAG (r) CTGTTTCTTCAATAGTGGAGGATCCCC	377	35 cycles: 94°C 1 min 62°C 1 min 72°C 1 min
rs 3918332	(f) TTCCAGGTATATCTTCCTCTGCTGCAG (r) ATTCTGTCCTATAGTGGGTGAATGGAG	327	94°C 1 min 60°C 1 min 72°C 1 min
rs4150374	(f) CTGGAGTGCAGTGGTGAGAAC (r) CCTCAGGAGCAACACGACTTG	368	94°C 1 min 60°C 1 min 72°C 1 min

Agarose Gel Electrophoresis of DNA

DNA fragments were separated by electrophoresis through an agarose gel. Agarose was made up at 1-2% w/v in 1 x TBE buffer, and heated to dissolve. Ethidium bromide was added to cooled agarose to give a final concentration of 100ng/ml. Loading buffer was added to each DNA sample (1:5). Bioline HyperLadder I, a size marker, was routinely loaded alongside samples. DNA was visualised using a BIO-RAD Gel Doc 2000.

10 x TBE Buffer
0.9M Tris-HCl
0.9M boric acid
20mM EDTA, pH 8.0

5 x sample buffer
100mM EDTA
0.1% Bromophenol blue
20% Ficoll

Restriction Digests

All restriction enzymes were supplied by New England Biolabs. Restriction digests were carried out as described in the table below.

Restriction Digest Conditions

Gene/Exon	Enzyme		Digest Conditions
XPD exon 6	HinfI	PCR product 20µl 10X NEB buffer 2 5µl 20U HinfI 2µl H_2O 23µl	Digest at 37°C for at least 2 hours. Run 20µl on 2% regular agar gel.
XPD exon 22	FokI	PCR product 20µl 10X NEB buffer 4 5µl 4U FokI 1µl H_2O 24µl	Digest at 37°C for 2 hours Run 20µl on a 2% low melting point gel.
XPD exon 23	PstI	PCR product 20µl 10X NEB buffer 3 5µl 20U PstI 1µl H_2O 24µl	Digest at 37°C for at least 2 hours. Run 20µl on a 2% low melting point gel.
ERCC1 exon 4	BsrDI	PCR product 20µl 10X NEB buffer 2 5µl 100X BSA 0.5µl 10U BsrDI 3µl H_2O 22µl	Digest at 65°C for at least 2 hours. Run 20µl on a 2% regular gel.
XPG exon 15	NlaIII	PCR product 20µl 10X NEB buffer 4 5µl 100X BSA 0.5µl 20U NlaIII 2µl H_2O 23µl	Digest at 37°C for at least 2 hours. Run 20µl on a 2% regular gel.
XPF exon 11	AlwNI	PCR product 20µl 10X NEB buffer 4 5µl 20U AlwNI 2µl H_2O 23µl	Digest at 37°C for at least 2 hours. Run 20µl on a 1.4% regular gel.
XRCC1 exon 10	MspI	PCR product 20µl 10X NEB buffer 2 5µl 50U MspI 2.5µl H_2O 22.5µl	Digest at 37°C for at least 2 hours. Run 20µl on a 2% low melting point gel.
XRCC3 exon 7	NlaIII	PCR product 20µl 10X NEB buffer 4 5µl 100X BSA 0.5µl 20U NlaIII 2µl H_2O 23ul	Digest at 37°C for at least 2 hours. Run 20µl on a 2% regular gel.
CKM 8	TaqI	PCR product 20µl 10X NEB buffer U 5µl 100X BSA	Digest at 65°C for at least 2 hours. Run 20µl on a 1% regular gel.

		0.5µl TaqI 1µl H_2O 24µl	
rs4150265	NcoI	PCR product 20µl 10X NEB buffer 4 5µl 20U NcoI 2µl H_2O 23µl	Digest at 37°C for at least 2 hours. Run 20µl on a 2% regular gel.
p53 codon 72	BstUI	PCR product 20µ 10X NEB buffer 2 2µl BstUI 5µl H_2O 23µl	Digest at 60°C for at least 2 hours. Run 20µl on a 2% low melting point gel.

SNaPshot analysis

The ABI PRISM SNaPshot Multiplex Kit from Applied Biosystems was used in conjunction with the ABI 310 Genetic Analyzer, located within the MRC Human Genetics Unit, Edinburgh, to genotype polymorphisms for which no restriction site existed. The multiplex kit is based on the dideoxy single-base extension of an unlabeled oligonucleotide primer. Each primer binds to a complimentary template in the presence of fluorescently labelled ddNTPs and AmpliTaq DNA Polymerase, FS. The polymerase extends the primer by one nucleotide, adding a single ddNTP to its 3' end. GeneScan Analysis Software from Applied Biosystems was then used to analyse polymorphisms.

Overview of SNaPshot technique

Template Preparation

- genomic DNA amplified by PCR
- removal of dNTPs and primers, by use of SAP and ExoI
- obtain purified template which contains target nucleotide, for example;

Target nucleotide

NNAGCATGCTCAATCGAATCCAANNNNN
NNTCGTACGAGTTAGCTTAGGTTNNNNN

Reaction Preparation - sample prepared for reaction, combining:

- Template DNA
- Primer, designed so as to stop immediately 5' to target nucleotide
- SNaPshot reaction mix

Thermocyling

Target nucleotide

- Denature template

NNAGCATGCTCAATCGAATCCAANNNNN

NNTCGTACGAGTTAGCTTAGGTTNNNNN

- Anneal unlabelled primer

TCGTACGAGTTAGCTTAGGT
NNAGCATGCTCAATCGAATCCAANNNNN

- Extend primer with target complementary ddNTP

dROX-labelled nucleotide

TCGTACGAGTTAGCTTAGGTT
NNAGCATGCTCAATCGAATCCAANNNNN

Post-Extension Treatment

- Remove unincorporated ddNTPs with SAP and denature

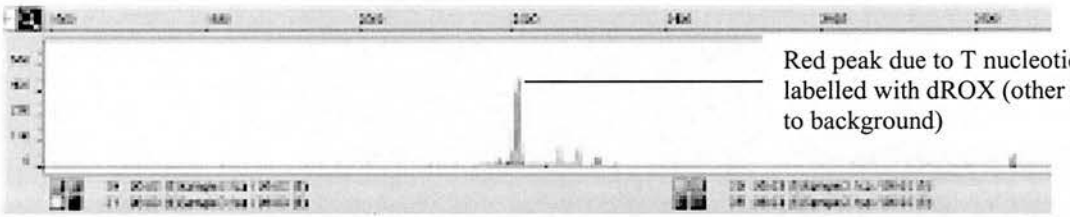
dROX-labelled nucleotide

TCGTACGAGTTAGCTTAGGTT

NNAGCATGCTCAATCGAATCCAANNNNN

GeneScan Analysis

- Electrophorese samples on ABI 310 machine
- Analyse data with GeneScan software



Red peak due to T nucleotide, which is labelled with dROX (other peaks due to background)

The fluorescent dyes assigned to the individual ddNTPs are as follows:

ddNTP	Dye Label	Colour of Analysed Data
A	dR6G	Green
C	dTAMRA	Black
G	dR110	Blue
T (U)	dROX	Red

PCR products were the starting point for the SNaPshot analysis, and were obtained as described above.

Preparation of PCR products.

Primers and unincorporated dNTPs were removed from the template to avoid interference with the subsequent primer-extension reaction. 15µl of PCR product was added to 5 units of shrimp alkaline phosphatase (SAP) (USB, 100 unit/ml) and 2 units of Exonuclease I (*Exo* I) (USB, 10 units/µl) then mixed thoroughly. The samples were incubated at 37 °C

for 60 minutes, then at 75 °C for 15 minutes to inactivate the enzymes. The samples were kept at 4 °C until the primer-extension reaction was set up.

SNaPshot Primer-Extension Reactions.

Control reactions for the primer-extension were included in the reaction kit. The positive control contained six distinct primers, with length of final products varying from 21nt to 61nt. The control template contained an amplicon from CEPH DNA. All 4 signal colours were represented. This control enabled approximate run times to be determined. A negative control reaction was run without control template DNA. In addition, a sample containing the primers designed specifically for analysis of each SNP was run, without template DNA, enabling any peaks produced by the primers to be eliminated from the final GeneScan analysis.

Primer-extension reactions carried out as below. 3µl of treated PCR products were added to the following master mix:

SNaPshot Multiplex Ready Reaction Mix	5µl
Pooled SNaPshot primers	1µl
dd H_2O	1µl

Oligonucleotide primers for the SNaPshot reactions were as follows:

rs3918332 (f) GGTATGTGCAGTTGGGTGCTAGCAAAT
(r) CAATGGCAGCATGATCAGGAGACA

rs4150374 (f) ATCTCCTGACCTCGTGATCC
(r) AGCACTCTGGGAGGCCAAGGTGGG

The reaction mix was kept on ice before putting it in the thermal cycler. The conditions were as follows:

25 cycles of:
Rapid thermal ramp to 96 °C
96 °C for 10 seconds
Rapid thermal ramp to 50 °C
50 °C for 5 seconds
Rapid thermal ramp to 60 °C

60 °C for 30 seconds

Samples were kept at 4 °C until post-extension treatment was carried out.

Post-Extension treatment.

Post-extension treatment removed unincorporated ddNTPs. If left untreated, the unincorporated [F]ddNTPs would co-migrate with the fragment of interest. Removal of the 5' phoshoryl groups by phosphatase treatment alters the migration of the unincorporated [F]ddNTPs and thus prohibits interference.

To conduct post-extension treatment 1 unit of SAP was added to the reaction mixture, mixed thoroughly, and incubated at 37° C for 60 minutes. Samples were then incubated at 75 °C for 15 minutes to deactivate the enzyme. The reactions were kept at 4 °C for up to 24 hours before electrophoresis on the 310 system.

Electrophoresis using ABI PRISM 310 Genetic Analyzer.

In order to denature samples, 1µl of each sample was placed in the well of a 96-well plate. 9µl deionised formamide was added, mixed briefly and quickly spun down. The samples were denatured by placing them at 95 °C for 5 minutes before being loaded on the 310 Genetic Analyzer. The POP-4 polymer was used, in conjunction with the GS POP-4 (1mL) E5 module. The E5 Run Module encodes the following parameters on the 310 instrument:

Parameter	**GS POP-4 (1mL) E5**
Injection time	5 seconds
Electrophoresis voltage	15kV
Collection time	24 minutes
EP voltage	15 kV
Heat plate temperature	60 °C
Syringe pump time	150 seconds
Preinjection EP	120 seconds

ABI PRISM GeneScan Analysis Software version 3.1 was used to analyse data obtained after the samples had undergone electrophoresis.

Statistical Analysis

For each polymorphism the erythemal response to each dose of UVR was plotted against individual genotypes. Note some values are superimposed on others, a limitation of the software used. Analysis of variance (One way, unstacked) tests were carried out to determine if any association existed. Statistical analysis was carried out using the Minitab programme. The analysis of variance (ANOVA) test is a measure of the total variability in a set of data, which is given by the sum of squared differences of the observations from their overall mean, called the total sum of squares. This quantity is sub-divided into components that are identified with different causes of variation. After the contributions of all the specified sources of variation have been determined, the ermained, the residual sum of squares, or error sum of squares, is attributed to random variation. The mean square corresponding to the error sum of square (error SS) is often used as a measure of assessing the importance of the specified sources of variation, and the ratio of the error sum of squares compared to the total sum of squares value.

Lymphocyte preparation

Whole blood (10-20ml fresh blood/heparin) was collected from volunteers and lymphocytes extracted by Density Gradient Isolation within 2 hours.

Whole blood was diluted 1:1 with PBS, then carefully layered over 3ml Histopaque 1077 in a 50ml Falcon tube and centrifuged for at 400g for 20 minutes. Histopaque-1077 is a solution of polysucrose and sodium diatrizoate, which is adjusted to a density of 1.077+/-0.001g/ml. During centrifugation, erythrocytes and granulocytes are aggregated by polysucrose and sediment; whereas, lymphocytes remain at the plasma-Histopaque interface. The interface cells were removed using sterile disposable pastettes and diluted 1:1 with PBS before centrifugation at 400g for 10 minutes. The resulting pellet was resuspended in 10 ml PBS, and centrifugation carried out at 400g for 10 minutes. The

pellet was washed once more in lymphocyte-culture medium, without Concanavalin A, and centrifuged for a final time. The final pellet was resuspended in 12ml full culture medium, with 10μg/ml Concanavalin A. Cells were plated out in 60mm dishes.

Lymphocytes were cultured amd irradiated with UVR to investigate the effects of UVR on DNA content and apoptosis. Lymphocytes were chosen due to their relatively easy availability and short length of time required in culture.

Lymphocyte medium
44ml RPMI
0.5ml non-essential amino acids
0.5ml Sodium Pyruvate
5ml fetal calf serum
25μl β-mercaptoethanol
0.25ml penicillin/streptomycin
Total volume 50ml.

Lymphocyte culture

The lymphocytes were grown for 3 days in a Sanyo CO_2 incubator at a temperature of 37°C with 5% CO_2. Dishes were irradiated with 0J/m^2 (control dishes), 100J/m^2 or 400J/m^2 with UVC. A germicidal lamp, with an output at 254nm, was used. The UV source was suspended above the dishes at a height of 29cm. Lengthening the period of time the cells were exposed to the UVR increased UV doses. Following irradiation, 2mls fresh lymphocyte medium was added to each dish.

Cells were collected 2, 4, 8 or 24 hours after irradiation with UVR. Each dish was split into 2 to allow detection of apoptosis, and evaluation of the DNA content of cells.

Apoptosis Detection

The Annexin V-FITC Apoptosis Detection Kit was used to determine what percentage of cells underwent apoptosis after UV irradiation. Cells were collected either 2, 4, 8 or 24 hours after exposure to UVR by centrifugation at 500g for 5 minutes, then washed by resuspending in 500μl cold (4°C) 1x PBS and pelleted as above. Cells were then

resuspended in Annexin V Incubation Reagent, which was made up as below, at a concentration of $1x10^5$ to $1x10^6$ cells per 100µl, and incubated in the dark for 15 minutes at room temperature. 400µl 1x Binding buffer was then added to each sample. Samples were then analysed by flow cytometry within one hour.

Annexin V incubation Reagent – for 100µl

10 x Binding Buffer	10µl
Propidium Iodide	10µl
Annexin V-FITC	1µl
dH_20	79µl

DNA Content analysis

Flow cytometry was used to analysis the DNA content of the cultured cells.

Cells were collected by centrifugation at 1300g for 5 minutes. The pellet was resuspended in 100µl of citrate buffer and 450µl Solution A added. The samples were mixed gently by inversion, and incubated for 10 minutes at room temperature. 325µl Solution B was then added and the sample mixed and incubated as before. Finally, 250µl of the propidium iodide-containing Solution C were added, the sample mixed by inversion, and incubated on ice for 10 minutes. Samples were then analysed by flow cytometry.

Citrate Buffer
85.5g sucrose
11.76g trisodium citrate
50ml DMSO
make up to 1000ml with dH_2O.

adjust pH to 7.6

Stock Solution
2000mg trisodium citrate
121mg Tris
1044mg spermine tetrahydrochloride
2ml Nonidet P40
dissolve in dH_2O to 2000ml.

Adjust pH to 7.6

Solution A
15mg trypsin in 500ml stock solution. pH 7.6

Store frozen; bring to room temperature before use.

Solution B
250mg trypsin inhibitor
50mg ribonuclease A
in 500ml stock solution

Store frozen; bring to room temperature before use.

Solution C
208mg propidium iodide
500mg spermine tetrahydorochloride
in 500ml stock solution.

Store frozen; bring to 0°C before use.

XPD Exon 6.

Samples were genotyped for the XPD exon 6 polymorphism using PCR and an RFLP assay. A PCR product of 288 base pairs (bp) was obtained using the primers detailed in table 1 of the Methods chapter. The exon 6 polymorphism consists of an A to C change at position 22541 on the GenBank entry L47234, and does not lead to an amino acid substitution. A HinfI restriction site exists when an A is present. The CC homozygote does not contain this restriction site, and after digestion gives one fragment of 288bp. The AA homozygote yields two fragments of 206 and 82 bp, while the AC heterozygote gives all three fragments, of 288, 206 and 82 base pairs.

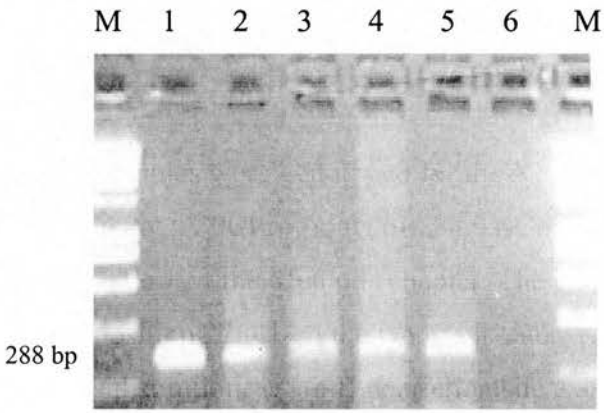


Figure 4.XPD exon 6 PCR products

Lanes 1-5, PCR products
Lane 6 – negative control
M – Bioline HyperLadder I, a size marker

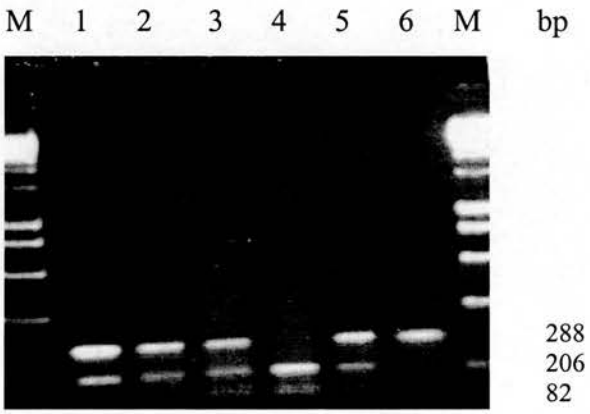


Figure 5.XPD exon 6 digestion products

Lanes 1, 2, 3, 5, AC
Lane 4, AA
Lane 6, CC

XPD exon 6 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
AA	13/74 (17.57%)	11/31 (35.48%)
AC	37/74 (50.00%)	15/31 (48.39%)
CC	24/74 (32.43%)	5/31 (16.13%)
Total	74 (100%)	31 (100%)

Genotype frequencies varied between the two study groups, but not statistically significantly (DF = 2, P-Value = 0.075). The heterozygous AC genotype had a similar frequency in both groups (50% in Group 1, 48.39% in Group 2), whereas the homozygous AA was the least common genotype in Group 1, but the second most frequent (after the heterozygotes) in Group 2. This may be due to chance, to the number of individuals in each group, or some factor that determined selection in each group. However, the exon 6 polymorphism has not previously been found to be associated with psoriasis or other atopic skin disease, nor is there evidence to suggest that such an association might be likely. As the presence of psoriasis in group 2 is the only common difference between the two study groups, it is likely that the genotype frequencies observed in the two study groups differ only by chance.

The erythemal response to each UVR dose was plotted against individual genotypes. Note that, as mentioned before, some values are superimposed upon others, which is a limitation of the Minitab software used. Analysis of variance tests were carried out, and data obtained given below.

Erythema induced by incremental doses of UVR by XPD exon 6 genotype in Group 1.

UVR on lower back, measured at 48 hours, n=74

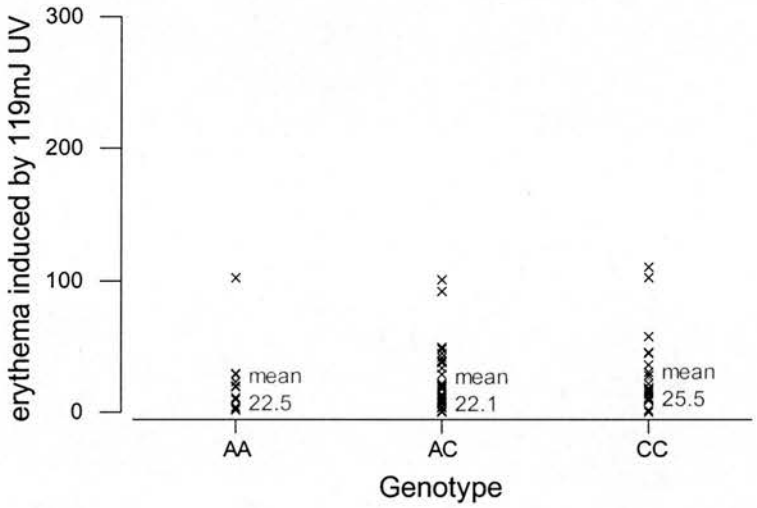


Figure 6. Erythema induced by 119mJ per cm^2 UV by XPD exon 6 genotype

UVR on lower back, measured at 48 hours, n=74.

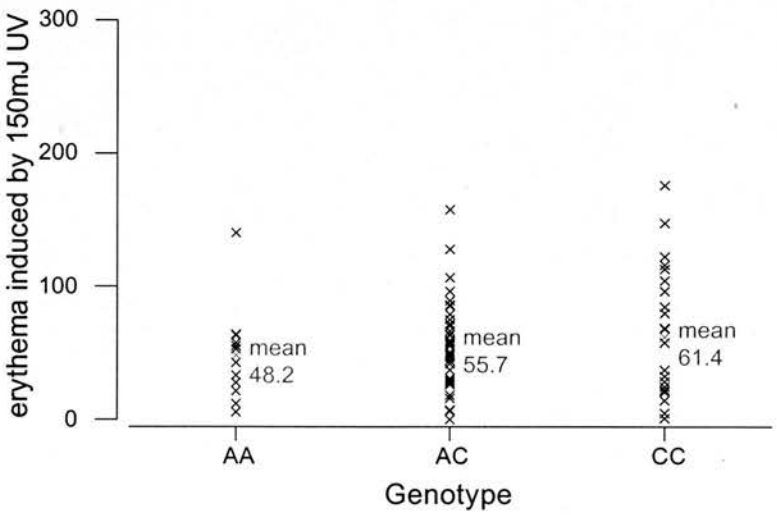


Figure 7. Erythema induced by 150mJ per cm^2 UV by XPD exon 6 genotype.

UVR on lower back, measured at 48 hours, n=74.

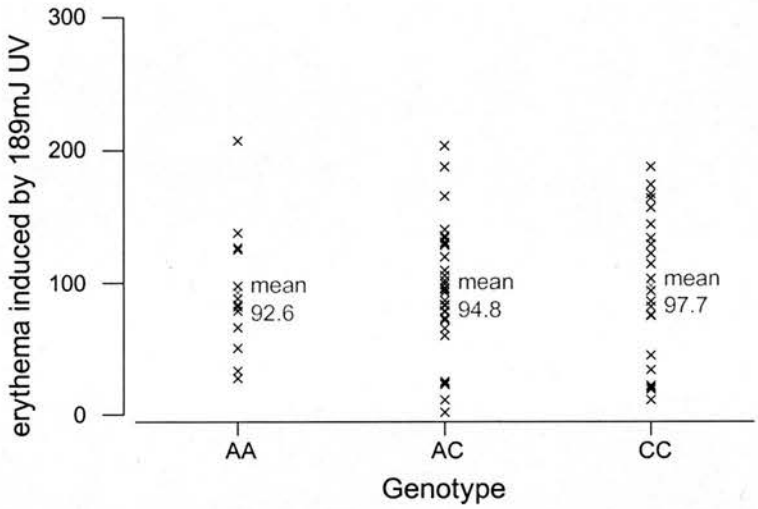


Figure 6. Erythema induced by 189mJ per cm^2 UV by XPD exon 6 genotype.
UVR on lower back, measured at 48 hours, n=74.

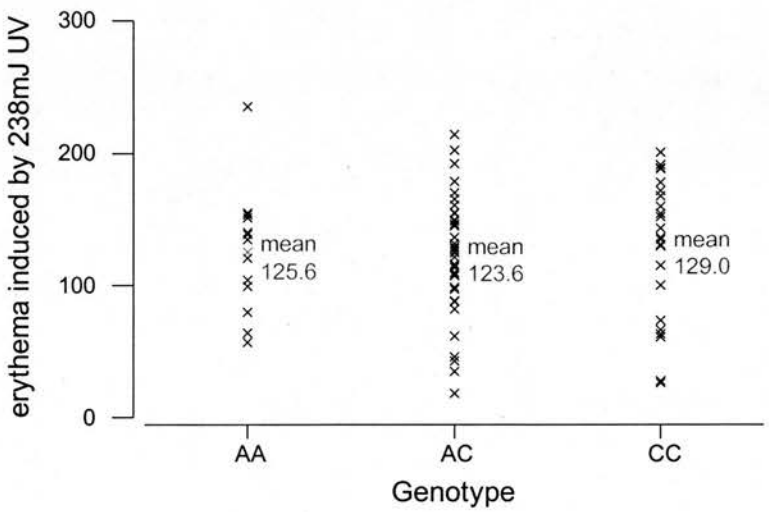


Figure 7. Erythema induced by 238mJ per cm^2 UV by XPD exon 6 genotype. UVR on lower back, measured at 48 hours, n=74.

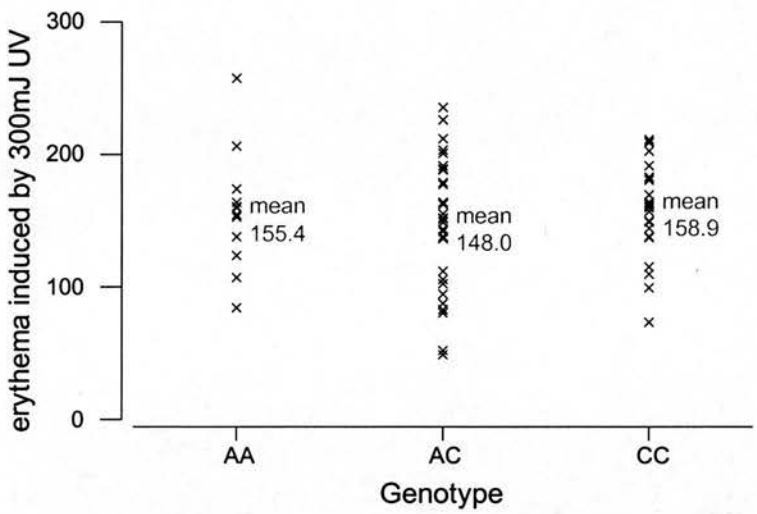


Figure 8. Erythema induced by 300mJ per cm^2 UV by XPD exon 6 genotype. UVR on lower back, measured at 48 hours, n=74.

Analysis of XPD exon 6 genotype and erythemal response to UVR for each dose for group 1.

Lower back, n=74

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	22.53	25.71	7.13
AC	37	22.07	23.44	3.85
CC	24	25.48	29.47	6.02

Analysis of variance of level of erythema from 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	178	89	0.13	0.876
Error	71	47683	672		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	48.20	33.78	9.37
AC	37	55.73	34.21	5.62
CC	24	61.41	48.93	9.99

Analysis of variance of level of erythema from 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1490	745	0.48	0.623
Error	71	110892	1562		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	92.6	48.4	13.4
AC	37	94.83	44.72	7.35
CC	24	97.7	53.6	10.9

Analysis of variance of erythema induced by 189mJ per cm^2 between genotypes:

Factor	2	242	121	0.05	0.950
Error	71	166075	2339		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	125.6	47.2	13.1
AC	37	123.64	45.84	7.54
CC	24	129.0	52.1	10.6

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	421	210	0.09	0.913
Error	71	164674	2319		
Total	73	165095			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	155.40	43.30	12.00
AC	37	148.00	46.01	7.56
CC	24	158.90	35.47	7.24

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1836	918	0.51	0.602
Error	71	127647	1798		
Total	73	129483			

Discussion

At none of the UV doses examined was there any significant difference in erythemal response to UV between the three genotypes, with P values ranging from 0.950 to 0.602.

The mean level of erythema was highest, at each dose, in the CC homozygotes, with a smaller spread of data points than the AC heterozygotes, but not than the AA group. This difference was not significant.

The XPD exon 6 genotype of the individuals had little effect on the variation of the levels of erythema at each UV dose examined in group 1. The sum of squares (SS) values of the factor and error were compared. The SS(Total) value (the SS(factor) plus SS(error)) accounts for all the variation, both between the genotype groupings, and within the genotype groupings. SS(factor) accounts for variation between the different genotypes, while SS(error) accounts for all external error, which is error within the different genotype groupings. On analysis of the two highest UV doses the volunteers were irradiated with, if the XPD exon 6 polymorphism was associated with UV-induced erythema, one might expect to see an enhanced difference between genotypes. It is apparent here that the error due to the different genotypes is insignificant when compared to that due to other factors. At 238mJ per cm^2, the SS(factor) value is 421, compared with the SS(error) value of 164674. At 300mJ per cm^2, the SS(factor) value is 1836, while the SS(error) value is 127647, accounting for the vast majority of the total error (SS(total) = 129483).

In order to measure the power of the above statistical analysis, retrospective power calculations were performed using the R Foundation for Statistical Computing software, which is downloadable from www.r-project.org. For analysis of the one-way ANOVA test, the level of variance between the populations (each different genotype) and the level of variance within the populations were analysed. The variation between populations is equivalent to the mean square (MS) of the factor in the ANOVA test, where factor is the genotype. The variation within populations is equivalent to the MS error value in the ANOVA test. The significance level was set to 0.05.

As the number of individuals with each genotype was unequal in this study, the ANOVA tests were unbalanced. In order to estimate power here, tests were performed assuming balanced groups, which would give the upper limit of power for this study, and for the least balanced the ANOVA could be, where all three groups contained the lowest number of individuals observed in the genotype groups. This test gave the lower limit of power of

the ANOVA calculations. Power calculations were performed for the lowest and highest UV doses in each group, in order to give an idea of power.

In group 1, a balanced ANOVA test would have had three groups with equal numbers of individuals in each, in this case, n=25. The genotype group with the fewest individuals was n=13, a power test carried out with 3 groups of 13 would give the lower limit of power for these samples. At 119mJ, the between variance value of 89 and the within variance value of 672 gave power values of 0.61 where n=25, and 0.33 where n=13. The true power therefore lies between these values. At 300mJ, the between variance value of 918 and within variance value of 1798 gave power values of 0.99 for a balanced ANOVA test, and 0.88 for the least balanced scenario. A power value of 1 is considered to be perfect power, and 0.8 considered highly desirable, therefore the power values obtained here are acceptable. The p values obtained in the ANOVA tests can be considered to be real.

Erythema induced by incremental doses of UVR by XPD exon 6 genotype for group 2.

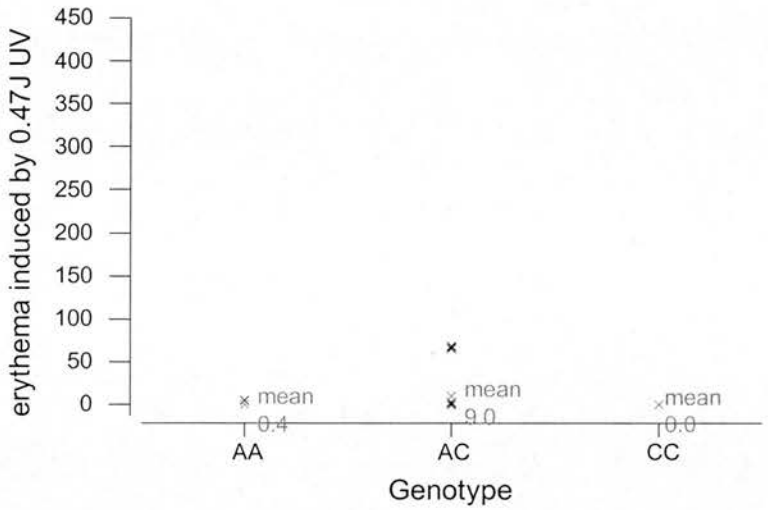


Figure 11. Erythema induced by 0.47J per cm^2 UV by XPD exon 6 genotype. UVR on inner forearm, measured at 24 hours, n=31.

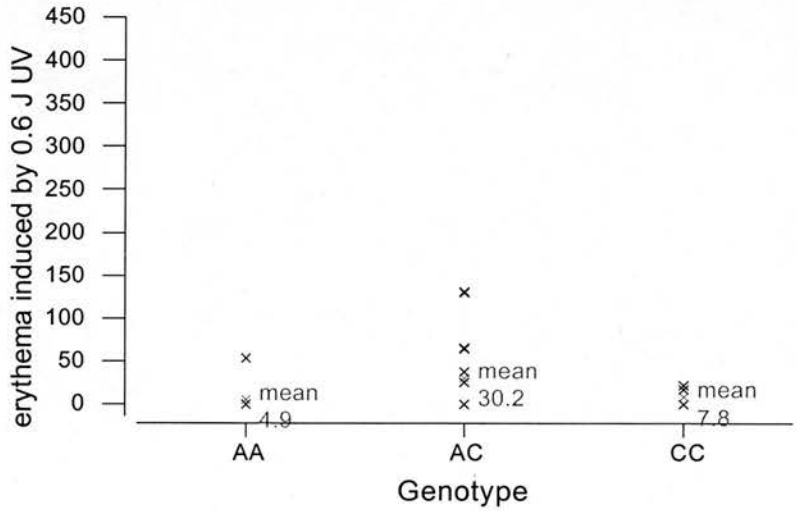


Figure 12. Erythema induced by 0.6J per cm^2 UV by XPD exon 6 genotype. UVR on inner forearm, measured at 24 hours, n=31.

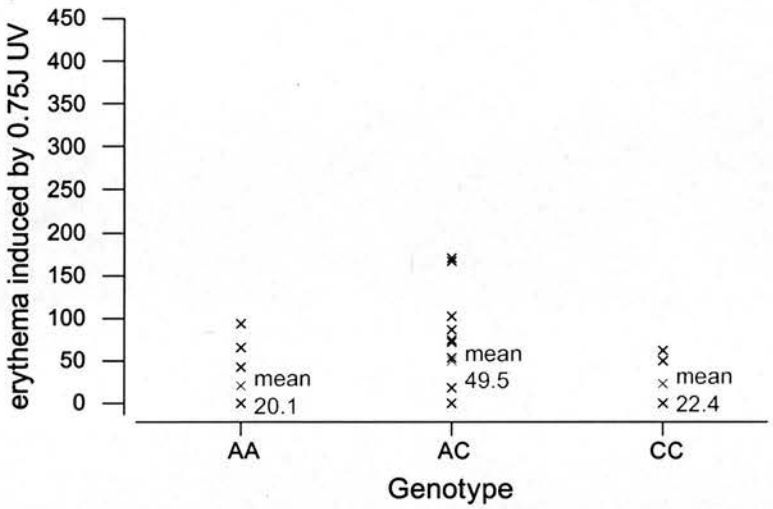


Figure 9. Erythema induced by 0.75J per cm^2 UV by XPD exon 6 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

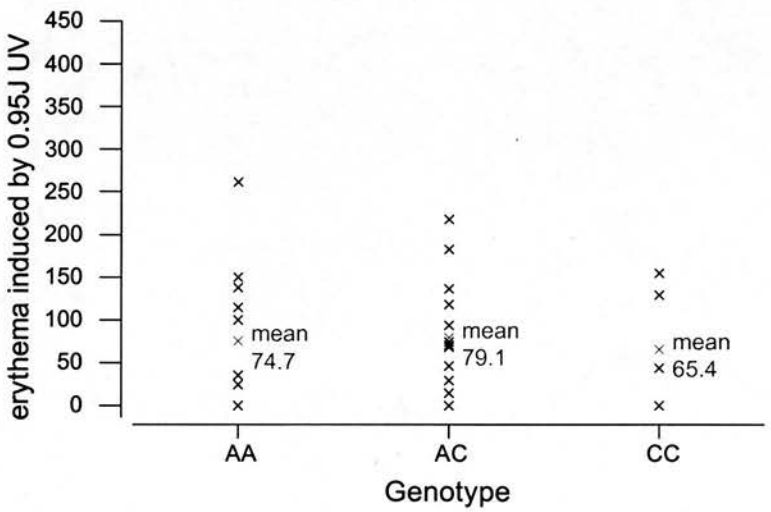


Figure 10. Erythema induced by 0.95J per cm^2 UV by XPD exon 6 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

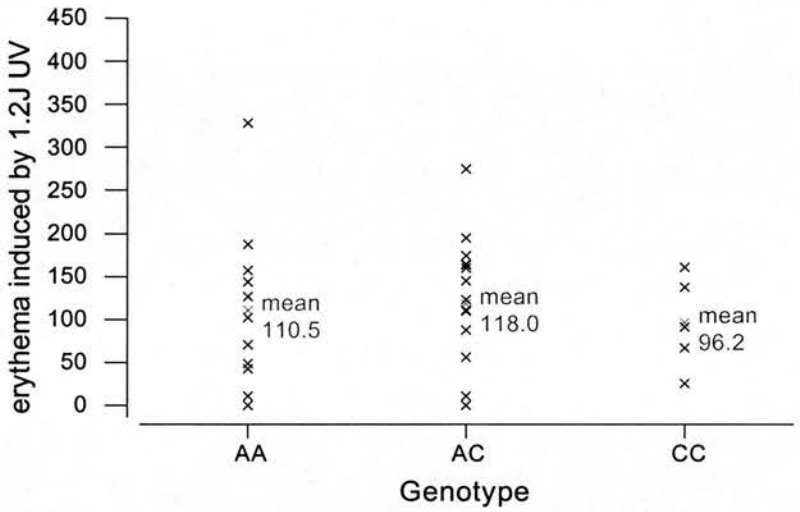


Figure 15. Erythema induced by 1.2J per cm^2 UV by XPD exon 6 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

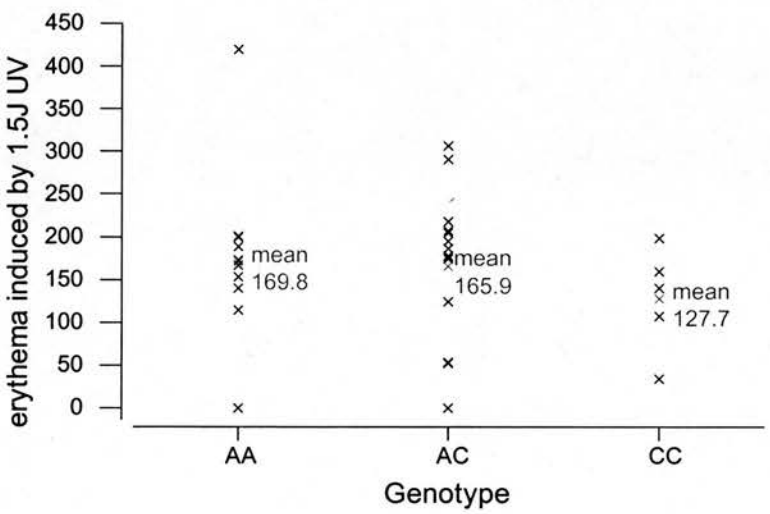


Figure 16. Erythema induced by 1.5J per cm^2 UV by XPD exon 6 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

Analysis of XPD exon 6 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	0.455	1.508	0.455
AC	15	9.00	23.36	6.03
CC	5	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	585	293	1.07	0.357
Error	28	7661	274		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	4.88	16.18	4.88
AC	15	30.2	46.9	12.1
CC	5	7.80	10.83	4.84

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	4642	2321	1.92	0.166
Error	28	33914	1211		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	20.06	32.77	9.88
AC	15	49.5	60.6	15.7
CC	5	22.4	31.0	13.8

Analysis of variance of erythema induced by 0.75J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	6385	3192	1.35	0.275

Error	28	66023	2358
Total	30	72408	

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	74.7	85.4	25.7
AC	15	79.1	68.1	17.6
CC	5	65.4	72.3	32.3

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	709	355	0.06	0.939
Error	28	158640	5666		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	110.5	94.3	28.4
AC	15	118.0	77.6	20.0
CC	5	96.2	53.7	24.0

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1816	908	0.14	0.872
Error	28	184772	6599		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	11	169.8	100.3	30.2
AC	15	165.9	84.4	21.8
CC	5	127.7	61.9	27.7

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P

Factor	2	6775	3388	0.44	0.649
Error	28	215667	7702		
Total	30	222442			

In group 2, again no significant association was observed at any of the UV doses examined and any genotype (P values ranged from 0.939 to 0.166). The heterozygous AC genotype tended to have higher mean levels of erythema than either homozygote, except at 1.5J per cm^2 UV.

In group 2, the total amount of variation at each UV dose examined was shown to be due to factors other than XPD exon 6 genotype. At 0.6J per cm^2, the UV dose with lowest p value (0.166) (which was not significant) the SS of total variation was 38555, of which the SS of variation due to genotype comprised very little, with a value of 4642. At the highest UV dose examined, variation due to the genotype was small when compared with the total variation. At all doses, variation due to other factors, the variation within each genotype group accounted for the majority of total variation.

Power calculations were carried out at 0.47J and 1.5J. A balanced ANOVA would have contained 10 individuals, and the least balanced 5. At 0.47J, the between variance value of 293 and within variance value of 274 gave a power of 0.98 where n=10, and 0.73 where n=5. At 1.5J the between variance value of 3388 and within variance value of 7702 gave powers of 0.71 where n=10, and 0.36 where n=5. This would suggest that not enough samples were looked at, as the true power level lies between 0.36 and 0.71, which is not high enough to accurately accept or reject the hypothesis.

XPD exon 6 Discussion

The reported association between the exon 6 polymorphism and melanoma (Tomescu *et al*, 2001) saw an over representation of the A allele at exon 6 in 28 patients with melanoma (aged under 50 years old) compared with 22 matched controls (Or 2.0, 95% CI 0.9-4.5, P=0.08). The CC genotype of the exon 6 polymorphism has been reported to be protective against basal cell carcinoma (BCC) (Dybdahl *et al*, 1999; Vogel *et al*, 2001).

In the complete study group, of 66 cases and 110 controls, individuals with an AA or AC genotype were reported to be at greater risk of BCC than those with the CC genotype (OR 1.9, 95% CI 0.96-3.75, p=0.0062). When only individuals with no family history of NMSC were analysed, individuals with the AA or AC genotype were found to be at greater risk of BCC (OR, 3.3, 95% CI 1.15-23.93, p=0.007). The effect of the exon 6 polymorphism is in the same direction as that reported by Tomescu and colleagues, who found the A allele to be over represented in patients with melanoma, suggesting the C allele offers more protection against cancers where UVR is the major causative agent than the A allele. Vogel and colleagues suggested that family history of BCC could override the effect of the XPD exon 6 polymorphism, explaining why formal significance was only observed in those with no family history of NMSC. It is, however, possible that individuals with a family history of NMSC were also carriers of another polymorphism involved with risk of developing BCC which those without the family history did not carry, and that the findings of the protective effect of the CC genotype in those without family history was either affected by another polymorphism, or due to chance. If the data obtained from this study were to support the findings of Tomescu *et al* and Vogel *et al*, it might be expected that the AA genotype would display higher levels of erythema than the AC genotype, and one would expect both these genotypes containing the A allele to be significantly more susceptible to UV (and hence have higher erythemal responses) than the CC homozygotes. This was not observed at any dose of UV examined in either study group. This does not necessarily disagree with the findings that the A allele is associated with melanoma, or that the C allele has a protective effect against basal cell carcinoma, as it might be involved in some step of carcinogenesis not directly related to UV, or be the marker for another polymorphism that is the causative change for melanoma. In order to resolve this, a greater number of individuals could be analysed, with the study group containing both those with a history of BCC and melanoma, and also healthy individuals with no prior skin cancers.

XPD exon 22

The XPD exon 22 polymorphism was genotyped using a PCR and RFLP assay. A PCR product of 229 bp was produced, which contained the polymorphism. The exon 22 polymorphism is a C to T change at position 35326 on the GenBank sequence L47234. A FokI restriction site exists in the presence of the T allele. The C allele is not digested by FokI, which gives a single fragment of 229bp. The T allele, which contains the restriction site, yields two fragments, of 135 and 94 bp. The heterozygous CT genotype therefore gives 3 fragments, the uncut 229 bp fragment, and the 135 and 94 base pair fragments observed in the presence of the FokI site.

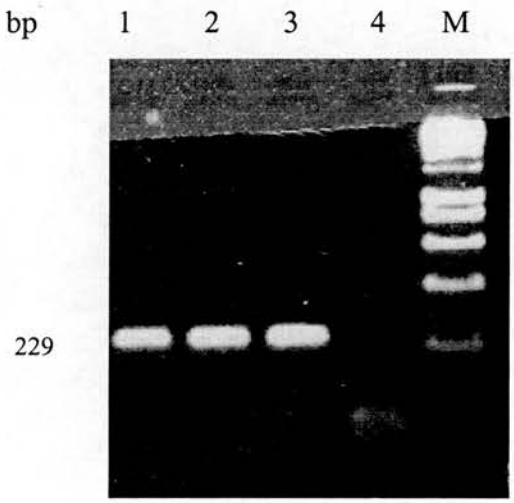


Figure 11XPD exon 22 PCR products

Lanes 1-3, PCR products
Lane 4 – negative control

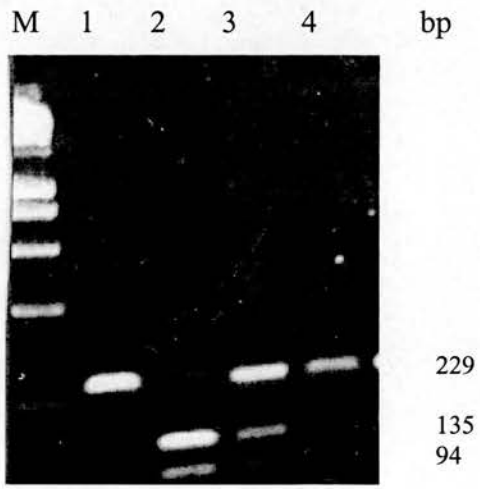


Figure 12XPD exon 22 digests

Lane 1, CC
Lane 2, TT
Lane 3, CT
Lane 4, undigested PCR product

XPD exon 22 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
CC	34/74 (45.945%)	16/31 (51.61%)
CT	34/74 (45.945%)	10/31 (32.26%)
TT	6/74 (8.11%)	5/31 (16.13%)
Total (100%)	74 (100%)	32 (100%)

The XPD exon 22 polymorphism showed a similar pattern of genotype frequency in both study groups. There was no significant genotype frequency between group 1 and group 2 (χ^2, DF = 2, P-Value = 0.291)

Erythema induced by incremental doses of UVR by XPD exon 22 genotype for group 1.

UVR on lower back, measured at 48 hours, n=74.

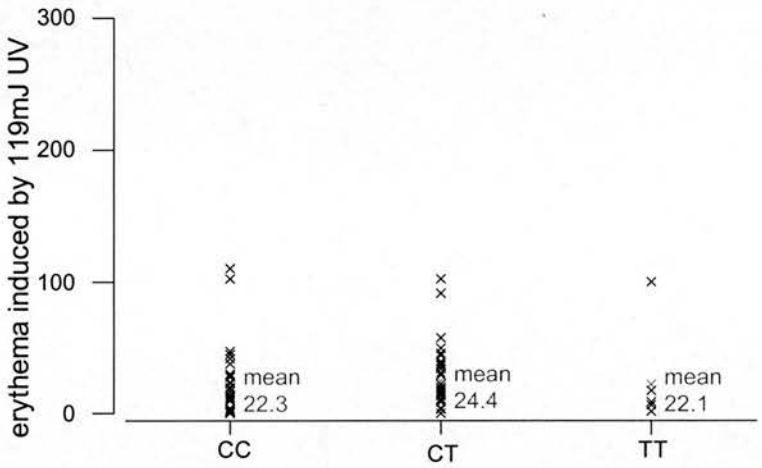


Figure 13 Erythema induced by 119mJ per cm^2 UV by XPD exon 22 genotype.
UVR on lower back, measured at 48 hours, n=74.

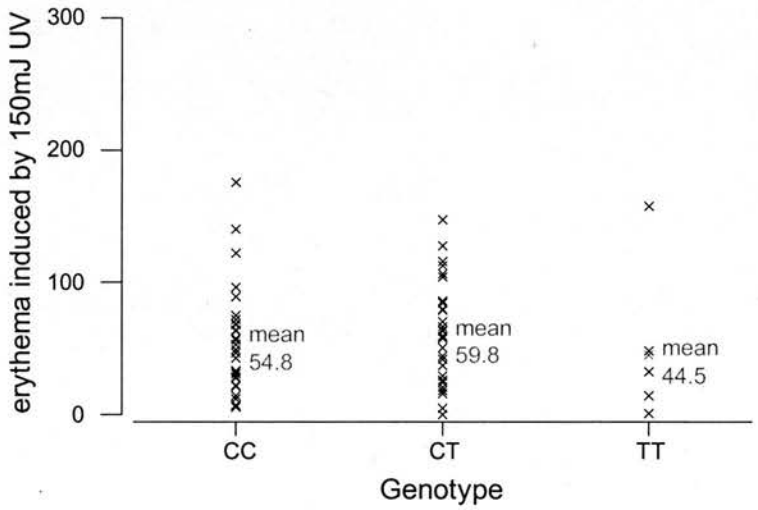


Figure 14. Erythema induced by 150mJ per cm^2 UV by XPD exon 22 genotype.
UVR on lower back, measured at 48 hours, n=74.

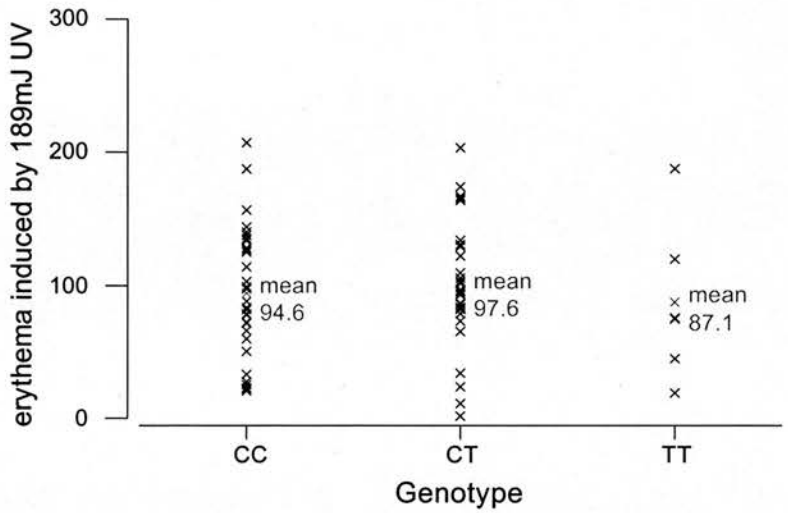


Figure 15. Erythema induced by 189mJ per cm^2 UV by XPD exon 22 genotype.
UVR on lower back, measured at 48 hours, n=74.

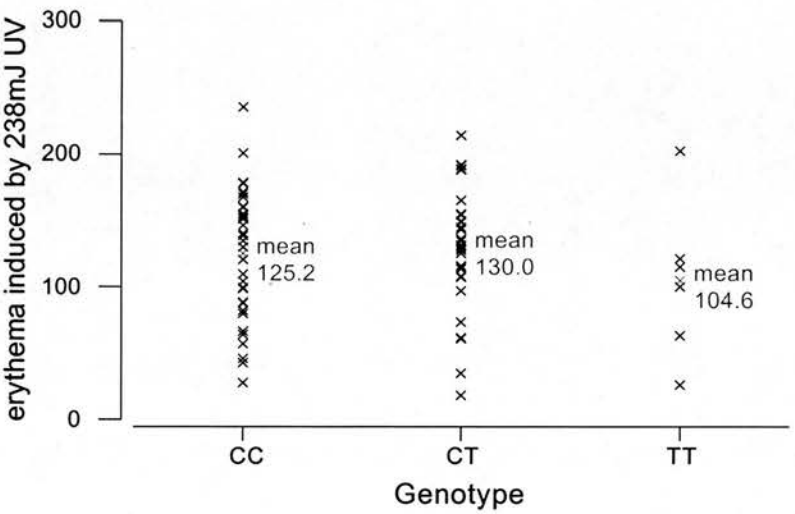


Figure 16. Erythema induced by 238mJ per cm^2 UV by XPD exon 22 genotype.
UVR on lower back, measured at 48 hours, n=74.

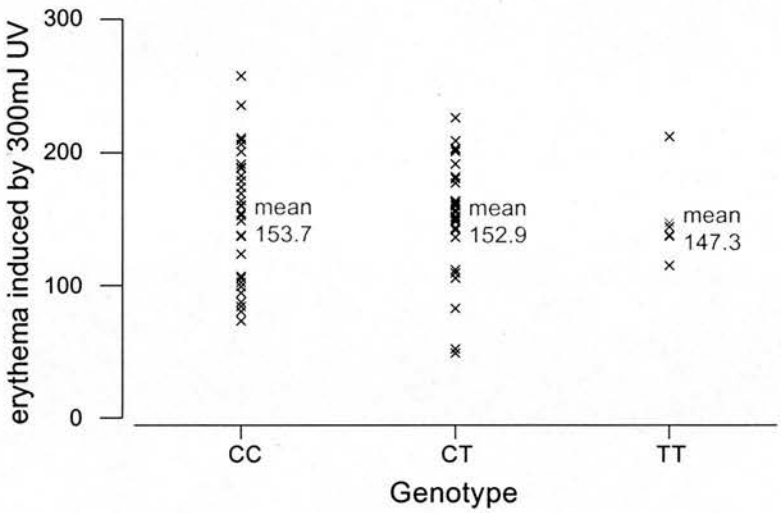


Figure 17. Erythema induced by 300mJ per cm^2 UV by XPD exon 22 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of XPD exon 22 genotype and erythemal response to UVR for each dose in group 1.

119mJper cm^2

Genotype	N	Mean	StDev	SE Mean
CC	34	22.27	25.09	4.30
CT	34	24.45	24.28	4.16
TT	6	22.1	38.8	15.8

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	89	45	0.07	0.936
Error	71	47772	673		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	34	54.76	38.47	6.60
CT	34	59.81	37.16	6.37
TT	6	44.5	57.7	23.6

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1332	666	0.43	0.655
Error	71	111050	1564		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	34	94.62	47.71	8.18
CT	34	97.57	46.93	8.05
TT	6	87.1	59.8	24.4

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	599	299	0.13	0.880
Error	71	165719	2334		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	34	125.19	49.48	8.49
CT	34	129.98	43.76	7.51
TT	6	104.6	59.7	24.4

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3308	1654	0.73	0.487
Error	71	161787	2279		
Total	73	165095			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	34	153.71	46.54	7.98
CT	34	152.94	39.80	6.83
TT	6	147.3	33.2	13.6

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	210	105	0.06	0.944
Error	71	129273	1821		
Total	73	129483			

No significant difference was observed between any genotype and response to UV at any dose examined (P values ranged from 0.944 to 0.487). The TT genotype had lower mean erythemal responses at each UV dose, but this was not significant. The number of TT homozygotes examined in group 1 was also far fewer than the number of individuals with CC or CT genotypes. Given the high p values observed in group 1, it is not surprising that almost all variation in levels of UV-induced erythema was due to factors other than the XPD exon 22 genotype. The exon 22 genotype accounted for very little of the total variation, at 238mJ per cm^2 (UV dose with lowest P value, 0.487) the SS(factor) equalled 3308, while SS(error) was 161787, accounting for almost all the total variation (SS(total) value 165095). At 300mJ per cm^2, where the p value was 0.944 (the highest observed in

this group for XPD exon 22 genotype) the XPD exon 22 genotype accounted for almost none of the total variation (SS(factor)), with a value of only 210, compared with the SS(error) value of 129273.

A balanced ANOVA test would have had n=25, where n is the number of individuals in each group, and the least balanced would be where n=6. At 119mJ, the between variance value of 45 and within variance value of 673 gave power values of 0.34 and 0.10 respectively. At 300mJ, the between variance value of 105 and within variance value of 1821 gave power values of 0.30 and 0.09. The power here is too low to able to accept or reject the hypothesis with confidence; therefore a larger study group is required.

Erythema induced by incremental doses of UVR by XPD exon 22 genotype in group 2.

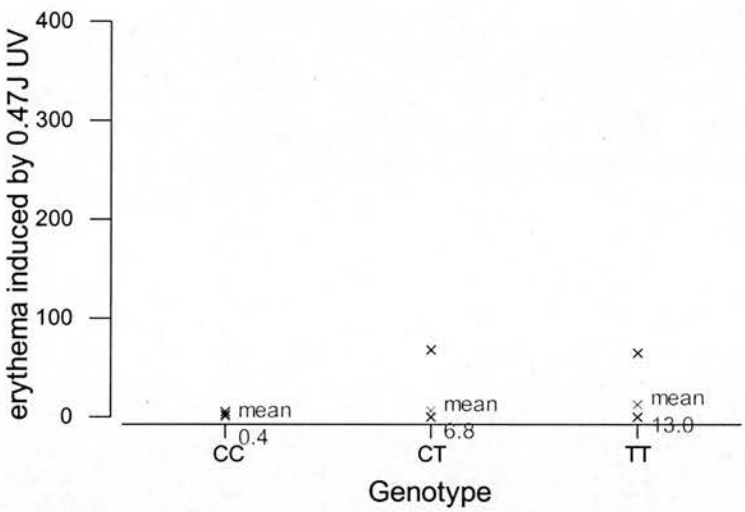


Figure 24. Erythema induced by 0.47J per cm^2 by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

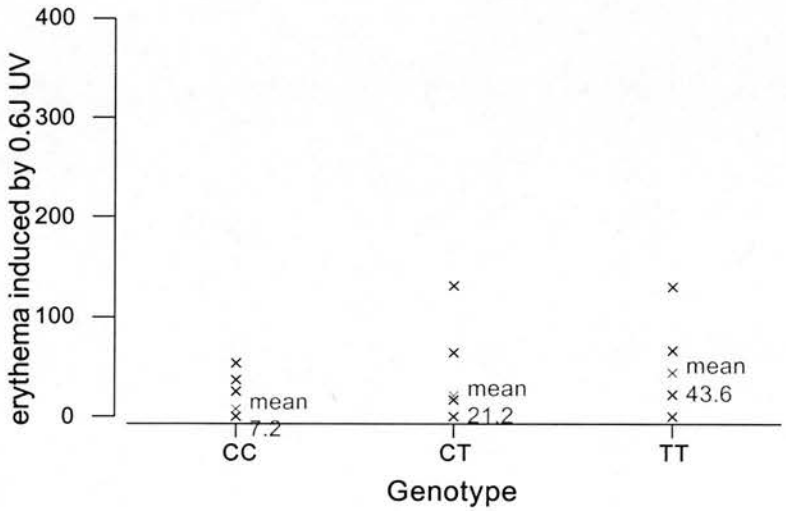


Figure 25. Erythema induced by 0.6J per cm^2 UV by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

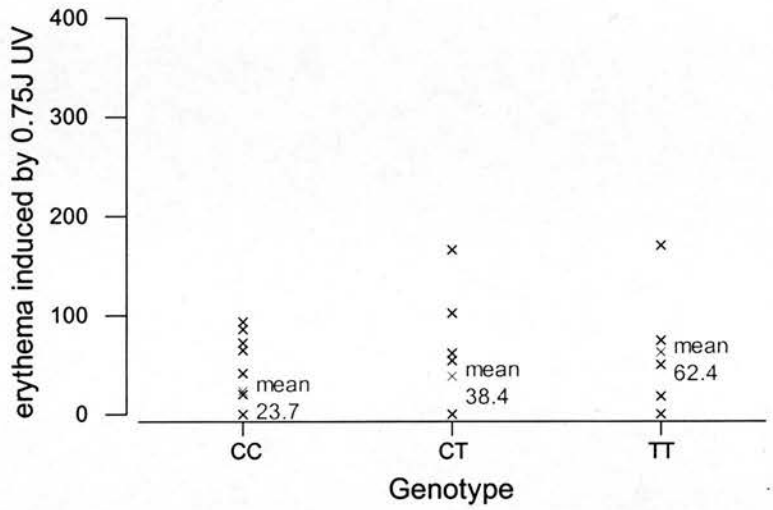


Figure 26 Erythema induced by 0.75J per cm^2 UV by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

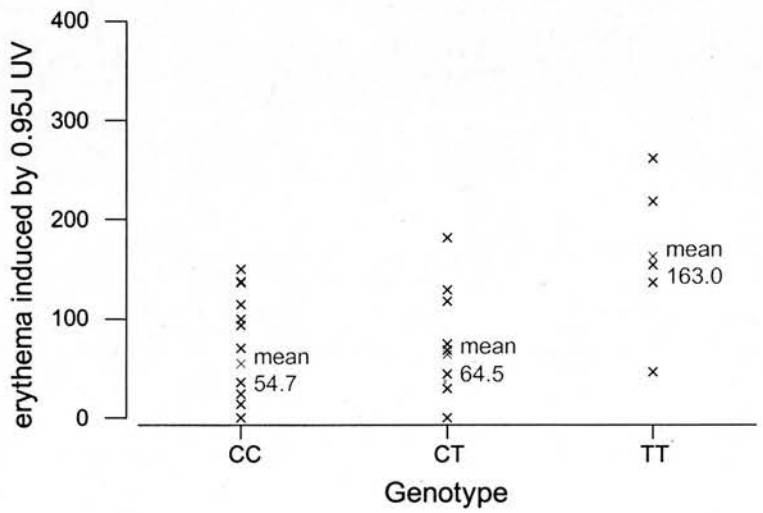


Figure 27 Erythema induced by 0.95J per cm^2 UV by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31

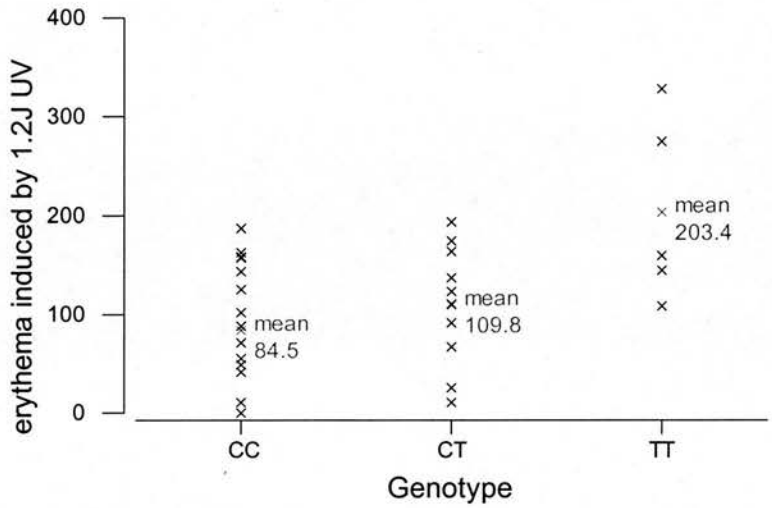


Figure 28 Erythema induced by 1.2J per cm^2 UV by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

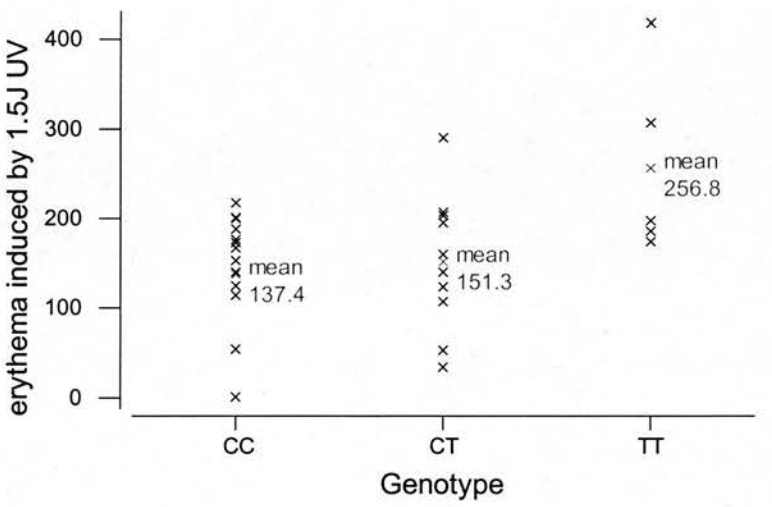


Figure 29. Erythema induced by 1.5J per cm^2 UV by XPD exon 22 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

Analysis of XPD exon 22 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	0.438	1.315	0.329
CT	10	6.80	21.50	6.80
TT	5	13.0	29.1	13.0

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	678	339	1.25	0.301
Error	28	7568	270		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	7.23	16.41	4.10
CT	10	21.2	43.5	13.8
TT	5	43.6	55.3	24.7

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	5230	2615	2.20	0.130
Error	28	33325	1190		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	23.67	35.38	8.85
CT	10	38.4	57.7	18.2
TT	5	62.4	66.6	29.8

Analysis of variance of erythema induced by 0.75J per cm^2 between genotypes:

Source	DF	SS	MS	F	P
Factor	2	5921	2960	1.25	0.303

Error	28	66487	2375
Total	30	72408	

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	54.7	58.2	14.5
CT	10	64.5	62.4	19.7
TT	5	163.0	82.4	36.8

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	46413	23207	5.75	0.008
Error	28	112936	4033		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	84.5	65.2	16.3
CT	10	109.8	61.5	19.4
TT	5	203.4	93.4	41.7

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	53901	26950	5.69	0.008
Error	28	132687	4739		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	16	137.4	67.7	16.9
CT	10	151.3	77.3	24.5
TT	5	256.8	105.1	47.0

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P

Factor	2	55759	27879	4.68	0.018
Error	28	166683	5953		
Total	30	222442			

In group 2, the TT homozygotes displayed higher mean erythemal responses at each dose examined. Significant variation in response to UV was seen at the three highest UV doses examined, 0.95J per cm^2 (p= 0.008), 1.2J per cm^2 (p=0.008) and 1.5J per cm^2 (p=0.018), although errors were wide. Although there was significant variation in level of erythemal response to UVB by genotype at 0.95, 1.2 and 1.5J per cm^2 UV, the variation due to genotype was still considerably less than the variation within the genotype groupings, which was variation due to other factors. At 0.95 and 1.2J per cm^2, where the p value was 0.008, the majority of variation was still due to factors other than the genotype, with SS(factor) values of 46413 for 0.95J per cm^2 and 53901 for 1.2J per cm^2 compared with the SS(error) values of 112936 and 132687 for 0.95 and 1.2J per cm^2 respectively. At 1.5J per cm^2 UV, (p value 0.018) the SS(total) value was 222442, of which only 5579 was accounted for by the XPD exon 22 genotype. The p values for group 2 alone might suggest an association between an increased susceptibility to UV (with higher erythemal response) and the TT genotype. However, this is not in agreement with the larger group 1, where no significant association between any genotype and level of UV-induced erythema was observed, and the trend (although not significant) was for the TT genotype to have lower levels of erythema.

This may be due to chance, or may be some peculiarity due to the make up of the study groups. Group 2 consisted of patients who were about to undergo phototherapy for psoriasis, the association seen in group 2 might be some artefact of this, or, more likely, due to chance.

Tomescu *et al* (2001) found the exon 22 polymorphism of XPD to be associated with melanoma in individuals under 50 years old. They found the C allele to be over represented in melanoma patients, compared with matched controls. From this, one would expect the CC genotype to have higher erythemal responses, which is not seen in group 2, and is not significant in group 1. The small number of individuals studied by

Tomescu *et al* might have exacerbated any artefacts, which lead to the finding of an association between the polymorphism and melanoma. The different data seen in the two independent study groups described here suggests that the association seen at the higher UV doses in group 2 is due to chance. The low power values obtained suggest a need for larger sample sizes in order to be confident there is no association between the XPD exon 22 genotype and erythema.

XPD exon 23

The exon 23 polymorphism in the only polymorphism in XPD examined in this study that leads to an amino acid change. An A to C change at position 35931 on the GenBank entry L47234 results in a non-synonymous amino acid change, from Lys to Gln.

A PCR-RFLP assay was used to genotype samples for the exon 23 polymorphism. PCR gave a 344 bp product, which contains the polymorphic site. The CC homozygote contains two PstI restriction sites, including one at position 35931 on GenBank entry L47234, giving fragments of 172, 110 and 62 bp after digestion. The AA homozygote only contains one restriction site, as in the presence of an A at the above position, no PstI restriction sited exists. This yields two fragments, of 234 and 110 bp. The heterozygous AC genotype gives four fragments, of 234, 172, 110 and 62 base pairs. (62bp fragment not seen on gel in Figure 30 below).

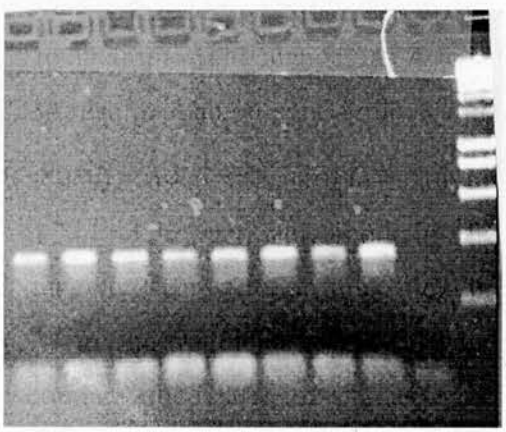


Figure 30, XPD exon 23 PCR products

Lanes 1-8, PCR products
Lane 9, negative control

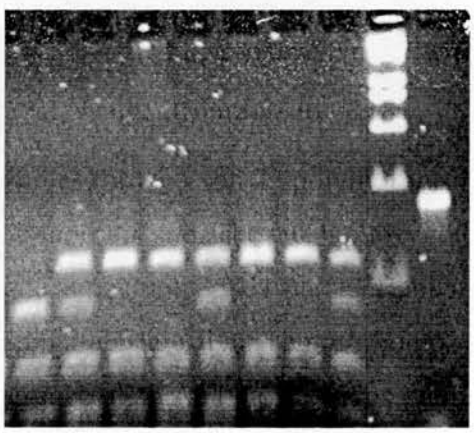


Figure 31 XPD exon 23 digests

Lane 1, CC
Lanes 2,5,8, AC
Lanes 3-4, 6-7, AA
Lane 9, undigested PCR product

XPD exon 23 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
AA	34/74 (45.95%)	17/31 (54.84%)
AC	33/74 (44.59%)	9/31 (29.03%)
CC	7/74 (9.46%)	5/74 (16.13%)
Total (100%)	74 (100%)	31 (100%)

The genotype frequencies observed in group 1 and group 2 followed a similar distribution. No significant difference was observed between the genotype frequencies in the two groups ($\chi 2$, DF = 2, P-Value = 0.282)

Erythema induced by incremental doses of UVR by XPD exon 23 genotype in group 1.

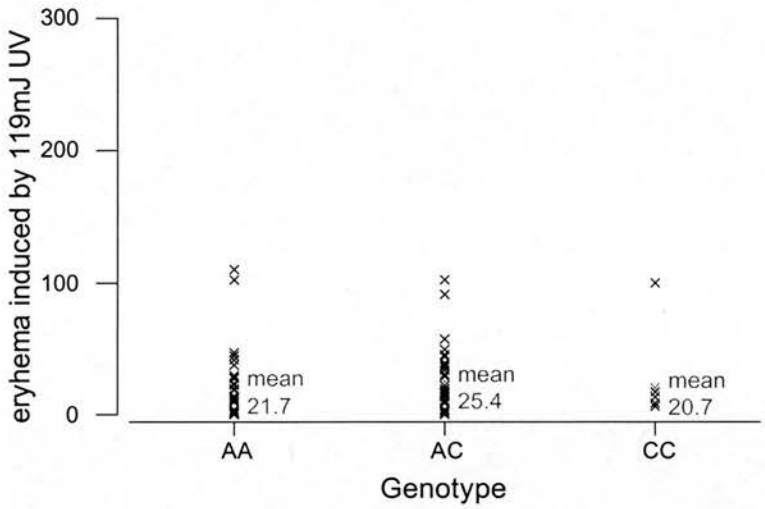


Figure 32 Erythema induced by 119mJ per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 48 hours, n=74.

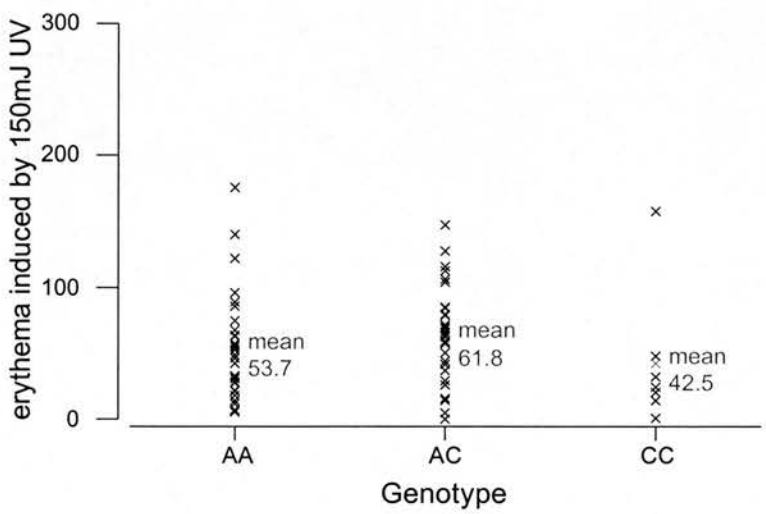


Figure 33 Erythema induced by 150mJ per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 48 hours, n=74.

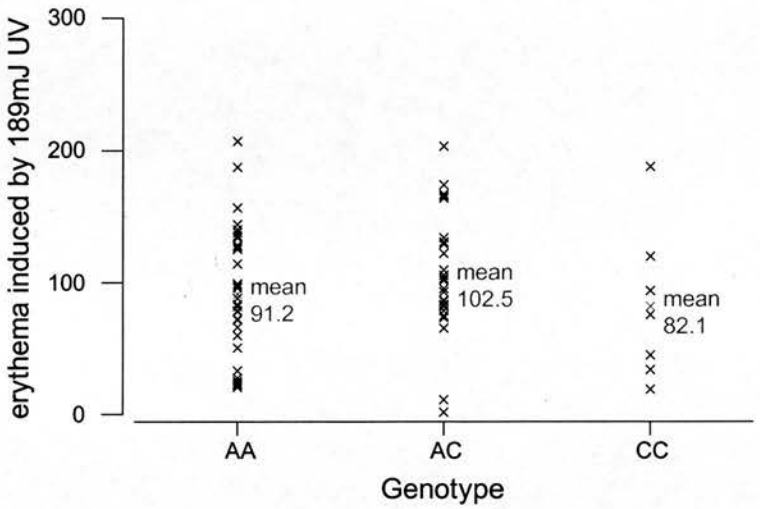


Figure 34 Erythema induced by 189mJ per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 48 hours, n=74.

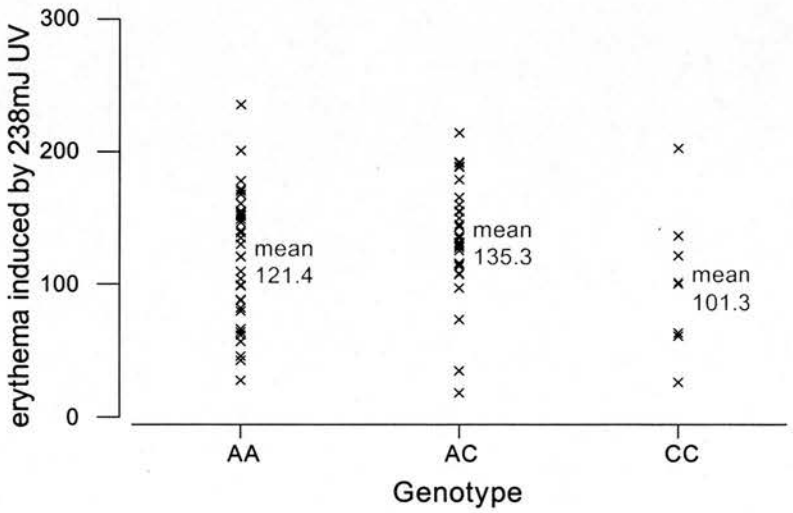


Figure 35. Erythema induced by 238mJ per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 48 hours, n=74.

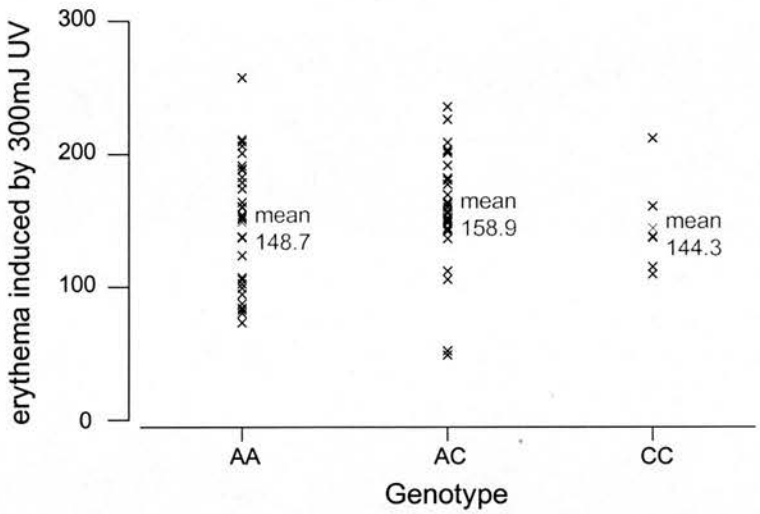


Figure 36. Erythema induced by 300mJ per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of XPD exon 23 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	34	21.74	25.45	4.36
AC	33	25.37	24.09	4.19
CC	7	20.7	35.7	13.5

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	272	136	0.20	0.817
Error	71	47589	670		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	34	53.71	39.17	6.72
AC	33	61.79	36.44	6.34
CC	7	42.5	52.8	19.9

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2556	1278	0.83	0.442
Error	71	109826	1547		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	34	91.16	48.67	8.35
AC	33	102.51	44.78	7.79
CC	7	82.1	58.4	22.1

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3525	1762	0.77	0.467
Error	71	162793	2293		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	34	121.41	49.51	8.49
AC	33	135.33	41.77	7.27
CC	7	101.3	58.5	22.1

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7849	3924	1.77	0.177
Error	71	157246	2215		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	34	148.68	45.64	7.83
AC	33	158.94	40.07	6.97
CC	7	144.3	34.3	13.0

Analysis of variance of erythema induced by genotype:

Source	DF	SS	MS	F	P
Factor	2	2324	1162	0.65	0.526
Error	71	127158	1791		
Total	73	129483			

In group 1, no significant association between any genotype and erythemal response was observed at any UV dose examined (p values from 0.817 to 0.177). The CC homozygote had lower mean levels of erythema at all doses, although errors were large, and this was not significant. The XPD exon 23 genotype explained very little of the overall amount of variation seen between levels of UV-induced erythema, as seen by low SS(factor) levels compared with the SS(error) and SS(total) values.

The number of individuals in each group if the ANOVA was balanced, and as least balanced as possible, would be n=25 and n=7 respectively. At 119mJ, the between variance value of 136 and within variance value of 670 gave power values of 0.80 where n=25 and 0.26 where n=7. At 300mJ, the between variance value of 1162 and within

variance value of 1791 gave power values of 0.99 and 0.70 respectively. The difference in power at the different UV doses suggests the sample size would need to be increased in order to ensure the p values obtained in the analysis of variance tests were accurate.

Erythema induced by incremental doses of UVR by XPD exon 23 genotype in group 2.

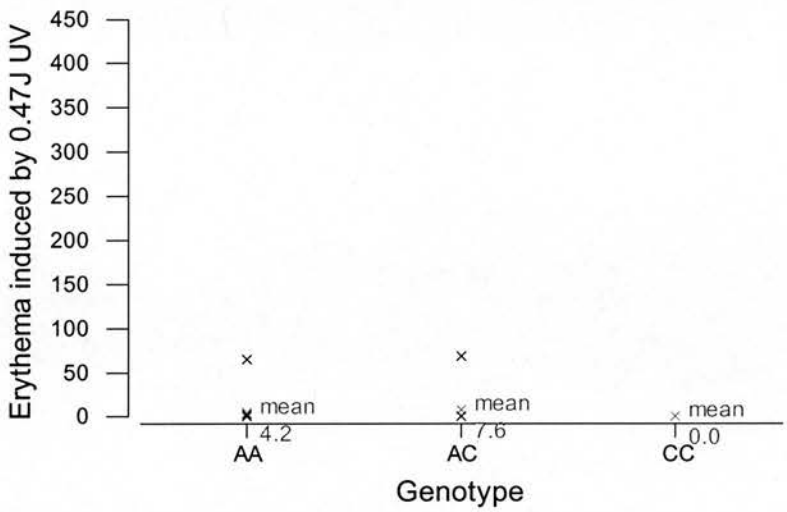


Figure 37. Erythema induced by 0.47J per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 24 hours, n=31.

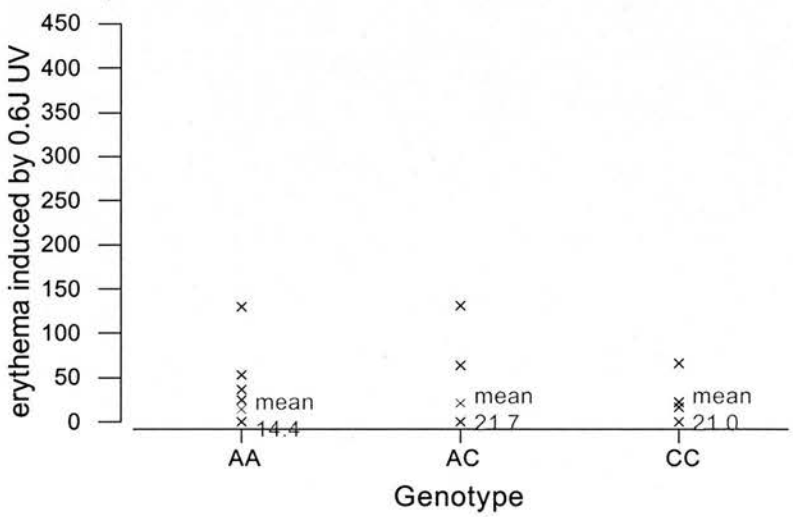


Figure 38. Erythema induced by 0.6J per cm^2 UV by XPD exon 23 genotype.

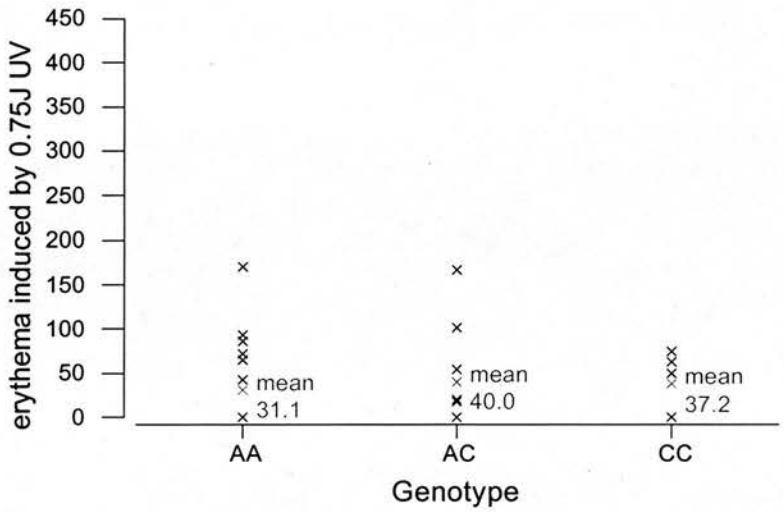


Figure 39 Erythema induced by 0.75J per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 24 hours, n=31.

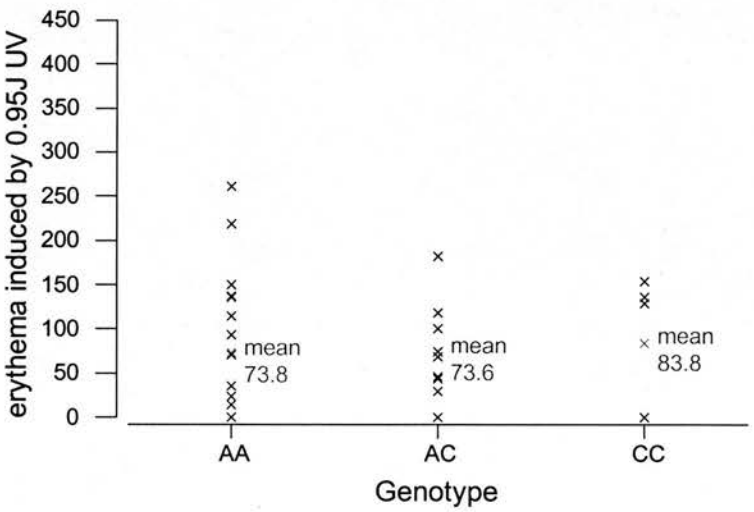


Figure 40 Erythema induced by 0.95J per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 24 hours, n=31.

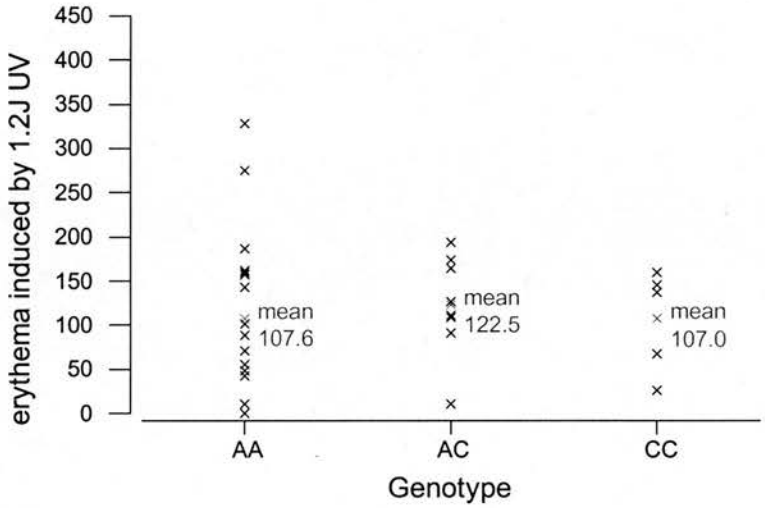


Figure 41 Erythema induced by 1.2J per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 24 hours, n=31.

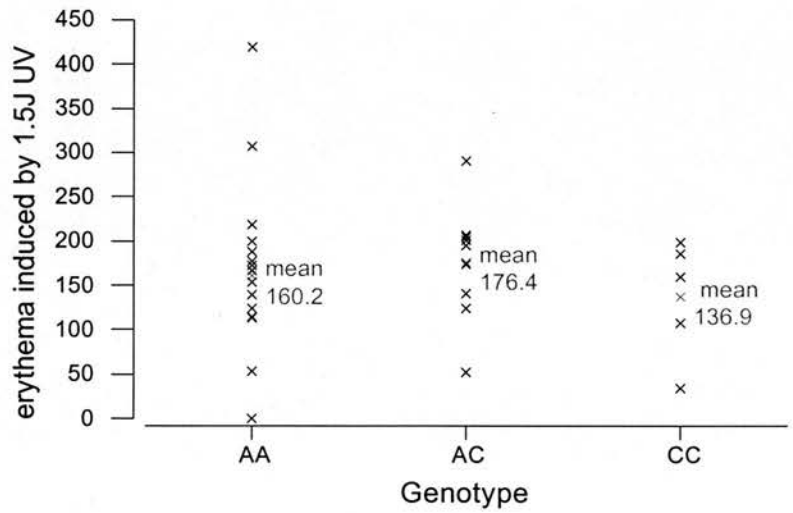


Figure 42 Erythema induced by 1.5J per cm^2 UV by XPD exon 23 genotype.
UVR on lower back, measured at 24 hours, n=31.

Analysis of XPD exon 23 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	4.24	15.71	3.81
AC	9	7.56	22.67	7.56
CC	5	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	186	93	0.32	0.726
Error	28	8059	288		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	14.45	33.75	8.19
AC	9	21.7	46.1	15.4
CC	5	21.0	27.0	12.1

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	375	188	0.14	0.872
Error	28	38180	1364		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	31.1	49.9	12.1
AC	9	40.0	58.3	19.4
CC	5	37.2	35.0	15.7

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	505	252	0.10	0.907
Error	28	71903	2568		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	73.8	83.6	20.3
AC	9	73.6	54.1	18.0
CC	5	83.8	77.0	34.5

Source	DF	SS	MS	F	P
Factor	2	428	214	0.04	0.963
Error	28	158922	5676		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	107.6	96.4	23.4
AC	9	122.5	53.9	18.0
CC	5	107.0	57.7	25.8

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1448	724	0.11	0.897
Error	28	185140	6612		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	17	160.2	101.2	24.5
AC	9	176.4	66.5	22.2
CC	5	136.9	67.3	30.1

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	5049	2525	0.33	0.725
Error	28	217393	7764		
Total	30	222442			

In group 2, again no significant association was observed between any genotype and erythemal response at any UV dose studied (p values ranging from 0.963 at 0.95J per cm^2 to 0.725 at 1.5J per cm^2). Variation in levels of erythema was in the main due to factors other than XPD exon 23 genotype, for example, at 1.5J per cm^2 UV, the genotype explained little of the total variation, with a SS(factor) value of only 5049, compared with the SS(total) of 217393, which made up the majority of the SS(total) value of 222442.

Power calculations for group 2 were carried out using n=10, which was equal to a balanced test, and n=5, the least balanced. 0.47J UV gave between variance values of 93 and within variance values of 288, giving power values of 0.57 when n=10 and 0.27 when n=5. At 1.5J the between variance value was 2525 and the within variance value 7764, which gave power values of 0.57 and 0.28, similar to those observed at the lower UV dose. Therefore, the ANOVA tests on this data are not very powerful, and require increased power, which could be achieved by a greater sample size, in order to be more confident in the p-values obtained.

Exon 23 Discussion

Previously, an association has been reported between the A allele of the exon 23 polymorphism and melanoma (Tomescu *et al*) OR 2-8, 95% CI 1.2-7.0, P=0.02. From this, it might be expected to see increased erythema in AA homozygotes than heterozygotes and CC homozygotes. The CC genotype did have lower erythema values in group 1, but this was not at all significant, and only seen at 1.2 and 1.5J per cm in group 2, where again it was not at all significant.

The lower level of chromosomal aberrations in cells from individuals with the CC genotype compared with those from AA and AC genotypes found by Vodicka and colleagues (2004) also suggests that the CC genotype may have some protective role in the development of cancer, as chromosomal aberrations are associated with onset of cancer. Vodicka and colleagues also report a higher level of irradiation specific DNA repair after gamma irradiation in lymphocytes with the CC genotype, compared to AA and AC genotypes (p=0.033), as measured by number of single stranded breaks in the DNA. The CC genotype did not display a protective role against UV-induced erythema, which would have been indicated by lower levels of erythema in both study groups,

suggesting that if it does provide some protective effect that lowers risk of cancers, this protection does not extend to protection from UV damage which could be linked to the ability of cells to carry out NER pathways. That the findings here are not in agreement with those of Tomescu *et al*, who found the A allele to be associated with melanoma, might be due to the lower sample sizes compared with the study in this thesis. Winsey *et al* (2000) also examined the exon 23 polymorphism for association with melanoma, and found no association between the two. This was a larger study, with 125 cases and 211 controls, compared with the 28 cases and 33 controls in the Tomescu study, which would suggest that the association seen by Tomescu and colleagues was due to chance. However, the use of cadavers as controls in the study by Winsey *et al* could be questioned. No mention is made of whether these individuals had had a history of melanoma, or any other skin cancer, or indeed whether this was investigated. If it were the case that any of the controls had previously had melanoma, their presence in the control group would affect the validity of the observed non-association between exon 23 and melanoma.

In their study investigating an association between the exon 23 polymorphism and lung cancer, Zhou *et al* (2002) saw no association between lung cancer and the XPG exon 23 polymorphism in their overall data set. When non-smokers were analysed alone, they observed an increased risk of lung cancer in individuals with the CC genotype when compared to those with an AA genotype (OR 2.0; 95% CI, 1.1-3.4. Zhou and colleagues suggested that as smoking reduces DNA repair capacity, any protective effect of the AA genotype wiped out by the carcinogens in tobacco in smokers. Although this study used a large number of cases and controls (1092 lung cancer patients, 1240 controls), the numbers of non-smokers were few in both sub-groups. Not surprisingly, amongst cases, the number of non-smokers (that is those who had never smoked) was only 73 out of 1092 (6.7%). The non-smokers composed more of the control group, but still only accounted for 434/1240 (35.0%). This is in effect a study of 73 cases and 434 controls, which could have skewed results due to the small number of cases compared with controls. Although most lung cancers are at least in part due to smoking, thus making it difficult to study large numbers of non-smoking, lung cancer patients, such a study would

be valuable in resolving this association. In a similar study, Hou *et al* (2002) analysed 185 Swedish lung cancer patients (97 smokers, 88 non-smokers) and 162 matched controls (83 smokers, 70 non-smokers), and found the presence of the C allele to be associated with increased risk of lung cancer in non-smokers only (OR 3.2; 95% CI 1.3-8.0). Other studies have, however, found no association between the exon 23 polymorphism and lung cancer risk. Park *et al* (2002) found no significant difference in the exon 23 genotype frequencies between Korean lung cancer patients and matched controls. David-Beabes and colleagues (2001) also found no association between exon 23, smoking status, and lung cancer risk. The evidence to both support, and disagree, with the association of exon 23 with lung cancer is inconclusive, but if such an association did exist, in affecting risk of lung cancer, it is reasonable to assume it would also affect risk of skin cancer, as DNA lesions induced by tobacco and UVR are both repaired by NER. The finding of an association with melanoma by Tomescu and colleagues is refuted in the larger study by Winsey *et al*. Data observed in this study implies no association between exon 23 and sensitivity to UVR, which is in agreement with no association being seen by Winsey *et al* in their melanoma study.

Of the three XPD polymorphisms studied here, only the exon 23 change affects the coding sequence (amino acid change of Lys to Gln). Tomescu *et al* found this association to be the strongest for this region of the gene, proposing that it could be causative. In this study, the exon 23 polymorphism was found to be no more associated with increased susceptibility to UV than exon 6 or exon 22. Lunn *et al* (2000) studied DNA repair capabilities of the two alleles of exon 23 and found the Lys allele to have reduced repair in an X-ray induced chromosome aberration assay. This is, however, ionising radiation, and the involvement of NER and XPD in repair of such damage is questionable. Dybdah *et al* (1999) found no difference in repair between the Lys and Gln alleles when using host cell reactivation and comet assays. This supports the findings here of no association between exon 23 and UV-induced erythemal response.

None of the polymorphisms of XPD studied here can be conclusively associated with an increased sensitivity to UVR. This is not in agreement with the study by Tomescu and

colleagues. Either susceptibility to UVR and development of melanoma are not related, or else the association seen between the polymorphisms of exon 6, 22 and 23 and melanoma are due to chance. It is well established that sensitivity to UVR is a risk facto r for the development of cutaneous malignancies, including melanoma. This, and the small number of individuals examined in the Tomescu study suggests that their reported association might be due to chance, as it is not backed up by the erythemal response data.

In the absence of further study, where larger study groups could be analysed, there is evidence to support an association of any of the polymorphisms examined in XPD (at exon 6, 22 and 23) and sensitivity to UVR. This suggests the findings of an association between these polymorphisms and melanoma (Tomescu *et al*) is due to chance.

CKM 8

A PCR-RFLP assay was used to genotype the CKM exon 8 polymorphism. PCR generated a 1099 bp product, which contained the polymorphic region. The polymorphism consists of a T to C chance at position 23977 on GenBank entry AC005781. In the presence of the C allele, a TaqI restriction site exists. The T allele is uncut, producing a single fragment of 1099 bp. The C allele yields two fragments, of 999 and 100 bp (not seen on gel below), and the heterozygous CT genotype this having 3 fragments after digestion; 1099, 999 and 100 bp (not seen on gel below).

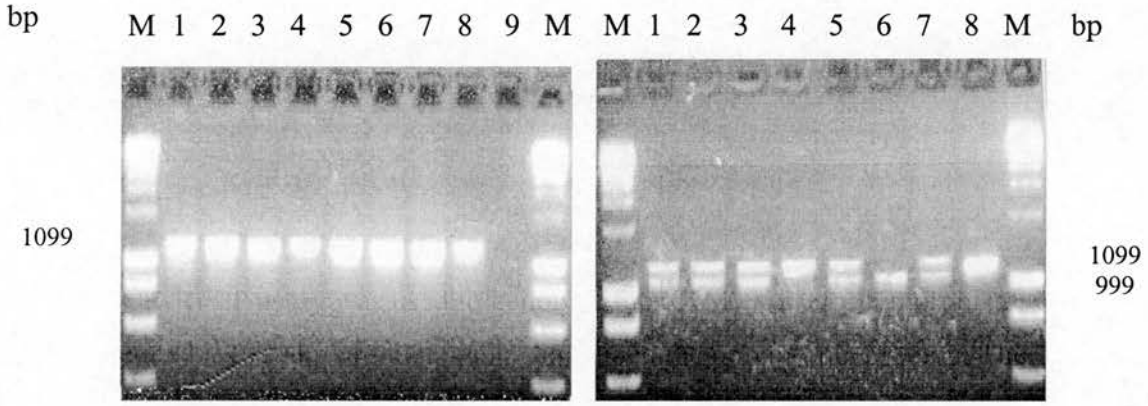


Figure 43, CKM exon 8 PCR

Lanes 1-8, PCR products
Lane 9, negative control

Figure 44 CKM exon 8 digest

Lanes 1-3, 5, 7, CT
Lanes 4, 8, TT
Lane 6, CC

It would not be expected to see any association between CKM exon 8 and susceptibility to UVR, as CKM has no role in the repair of UV-induced damage (or in any DNA repair pathway), and was included here to see if the reported association between XPD and melanoma (and hence susceptibility to UVR) by Tomescu *et al* extended to CKM, which is the nearest flanking marker to XPD. Tomescu and colleagues did not observe the reported association extending to CK M, but it this larger study group it is possible that if

an association was observed between any of the XPD polymorphisms it might extend to CKM. As no association was observed between any of the polymorphisms studied in XPD, no association was expected to be seen here in CKM.

CKM exon 8 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
TT	5/74 (6.76%)	2/31 (6.45%)
TC	28/74 (37.84%)	16/31 (51.%)
CC	41/74 (55.40%)	13/31 (41.94%)
Total (100%)	74 (100%)	31 (100%)

Genotype frequencies were similar between both study groups. Chi-square analysis confirmed that no significant genotype frequencies existed between the two groups (DF = 2, P-Value = 0.414). As the frequency of TT homozygotes was low, which very few being observed in either group, as well as analysing variance between all three genotypes, the TT homozygotes were grouped with the heterozygotes (TC) to determine variation between these and the CC homozygotes.

Erythema induced by incremental doses of UVR by CKM exon 8 genotype in group 1.

UV on lower back, measured at 48 hours, n=74.

Group 1

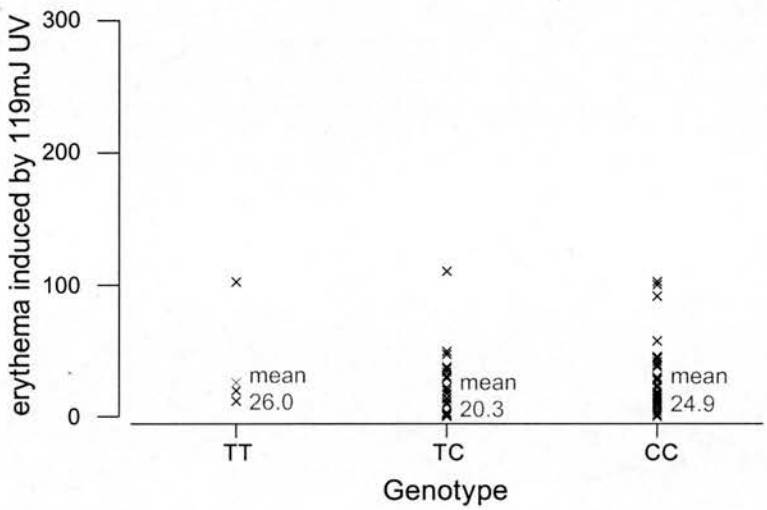


Figure 45. Erythema induced by 119mJ per cm^2 UV by CKM exon 8 genotype.
UVR on lower back, measured at 48 hours, n=74.

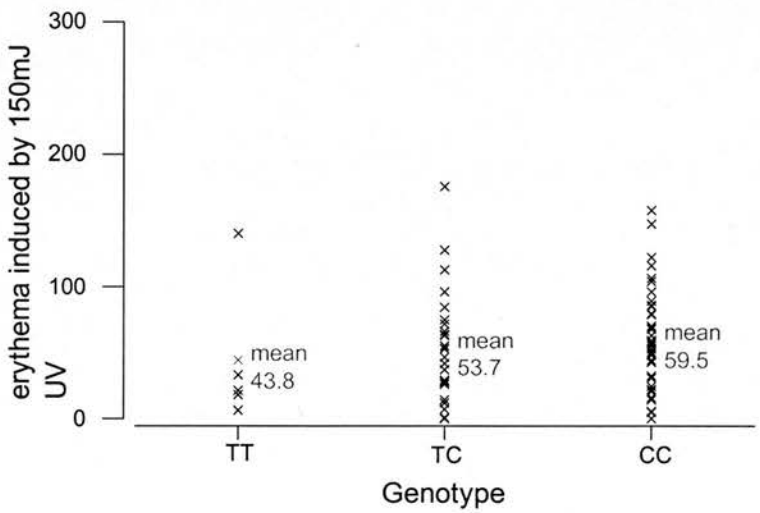


Figure 46. Erythema induced by 150mJ per cm^2 UV by CKM exon 8 genotype.
UVR on lower back, measured at 48 hours, n=74.

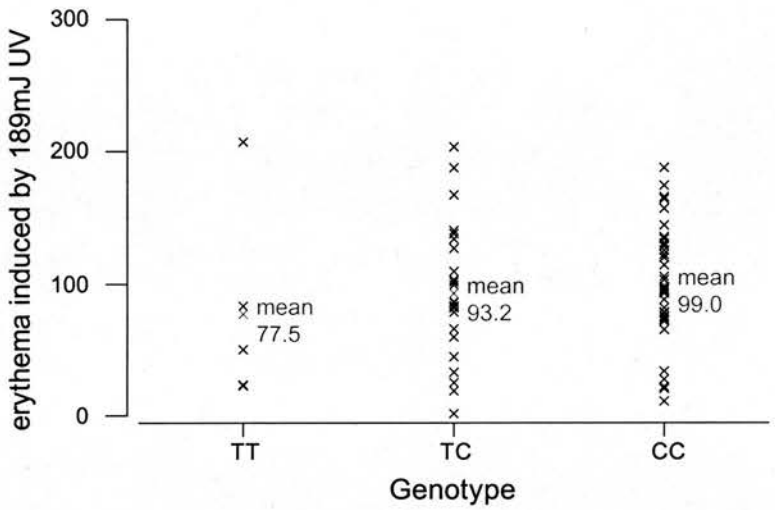


Figure 47 Erythema **induced by 189mJ per cm^2 UV by CKM exon 8 genotype.** UVR on lower back, measured at 48 hours, n=74.

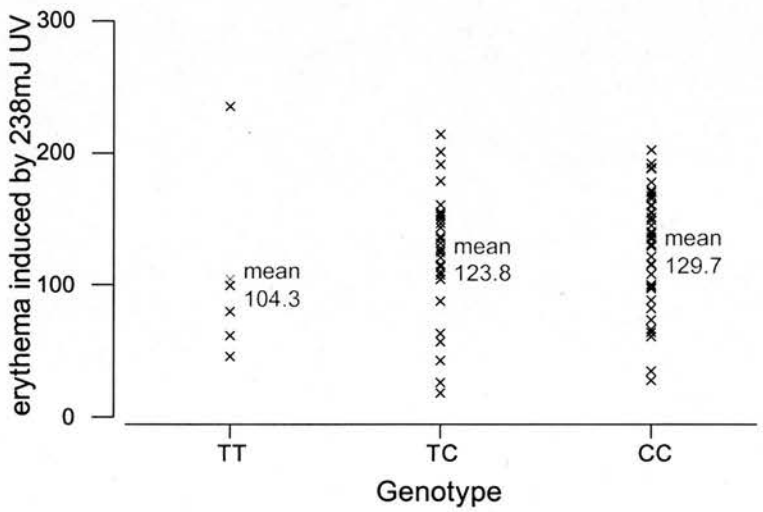


Figure 48. Erythema induced by 238mJ per cm^2 UV by CKM exon 8 genotype. UVR on lower back, measured at 48 hours, n=74.

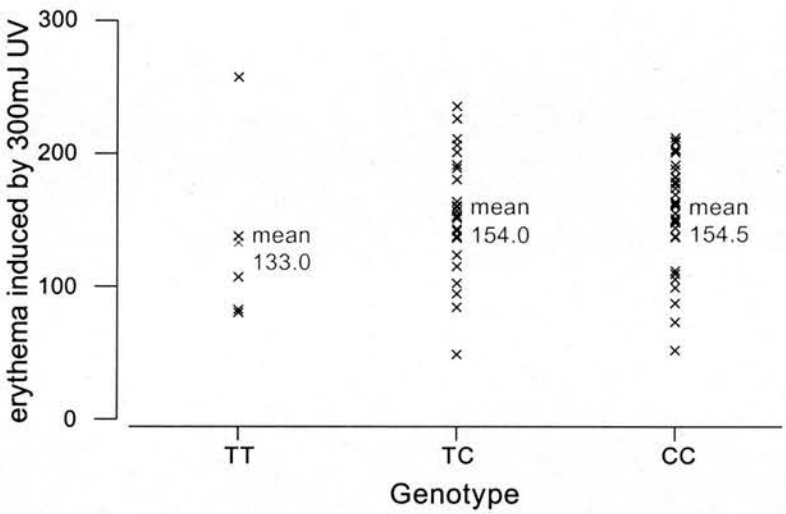


Figure 49. Erythema induced by 300mJ per cm^2 UV by CKM exon 8 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of CKM exon 8 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	5	26.0	43.4	19.4
TC	28	20.32	23.24	4.39
CC	41	24.93	25.17	3.93

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	395	197	0.30	0.745
Error	71	47466	669		
Total	73	47861			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	33	21.18	26.38	4.59
CC	41	24.93	25.17	3.93

Source	DF	SS	MS	F	P
Factor	1	256	256	0.39	0.536
Error	72	47605	661		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	5	43.8	54.9	24.5
TC	28	53.71	39.90	7.54
CC	41	59.51	37.40	5.84

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1391	695	0.44	0.643
Error	71	110991	1563		
Total	73	112382			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	33	52.21	41.63	7.25
CC	41	59.51	37.40	5.84

Source	DF	SS	MS	F	P
Factor	1	974	974	0.63	0.430
Error	72	111407	1547		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	5	77.5	76.6	34.2
TC	28	93.21	47.52	8.98
CC	41	99.01	44.61	6.97

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2278	1139	0.49	0.613
Error	71	164040	2310		
Total	73	166318			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	33	90.82	51.68	9.00
CC	41	99.01	44.61	6.97

Source	DF	SS	MS	F	P
Factor	1	1227	1227	0.53	0.467
Error	72	165091	2293		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	5	104.3	76.1	34.0
TC	28	123.78	49.29	9.32
CC	41	129.66	42.80	6.68

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3044	1522	0.67	0.517
Error	71	162051	2282		
Total					

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	33	120.82	53.15	9.25
CC	41	129.66	42.80	6.68

Source	DF	SS	MS	F	P
Factor	1	1428	1428	0.63	0.431
Error	72	163666	2273		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	5	133.0	73.3	32.8
TC	28	154.02	42.67	8.06
CC	41	154.46	37.66	5.88

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2114	1057	0.59	0.557
Error	71	127369	1794		
Total	73	129483			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	33	150.83	47.61	8.29
CC	41	154.46	37.66	5.88

Source	DF	SS	MS	F	P
Factor	1	240	240	0.13	0.716
Error	72	129243	1795		
Total	73	129483			

No association was seen between any genotype and susceptibility to UV at any dose. When analysing variation between each genotype, errors were wide for the TT genotype, due to small numbers. SS(factor) values were low at all UV doses examined when compared with the SS(error) values. This indicates the majority of variation is not attributable to the genotype of the individuals, and that the CKM exon 8 polymorphism is not associated with levels of UV-induced erythema in this study group.

If the analysis of variance was balanced, each group would contain the same number of individuals, which would be n=25. The least balance the test would be would be where n=5 in all groups. These values were used to give a range for the power of the tests. At 119mJ, the between variance value was 197, and the within variance 669. Power was 0.93 when n=25 and 0.25 when n=5. At 300mJ the between variance value was 1057 and within variance 1794, which gave powers of 0.99 and 0.47 when n=25 and 5 respectively. The true power will lie within this range, at the upper limit this would be acceptable, but greater power would enable it to be determined more confidently that the CKM genotype is not related to erythemal response.

Erythema induced by incremental doses of UVR by CKM exon 8 genotype in group 2.

Group 2

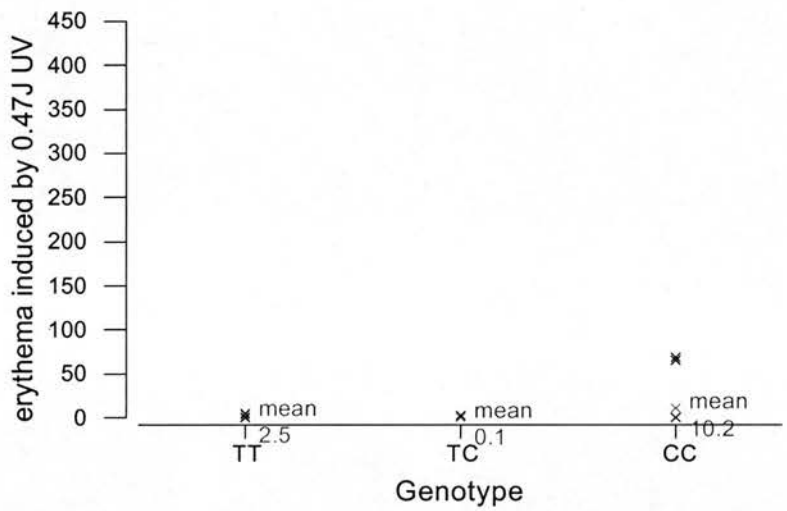


Figure 50. Erythema induced by 0.47J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

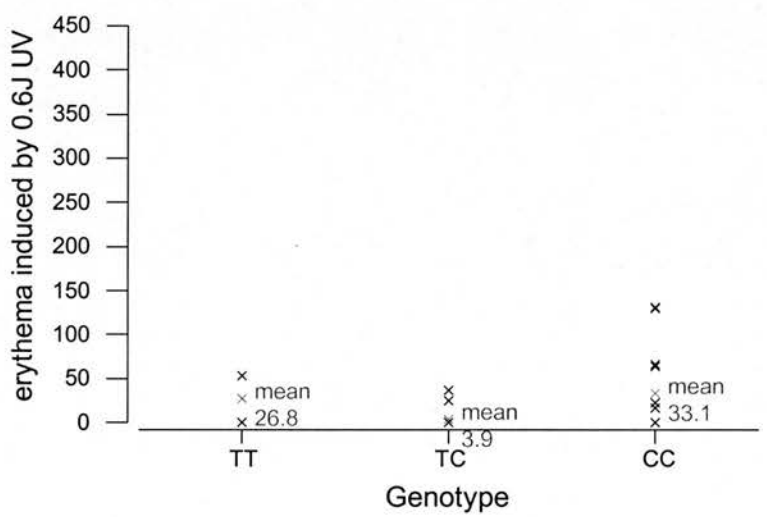


Figure 51 Erythema induced by 0.6J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

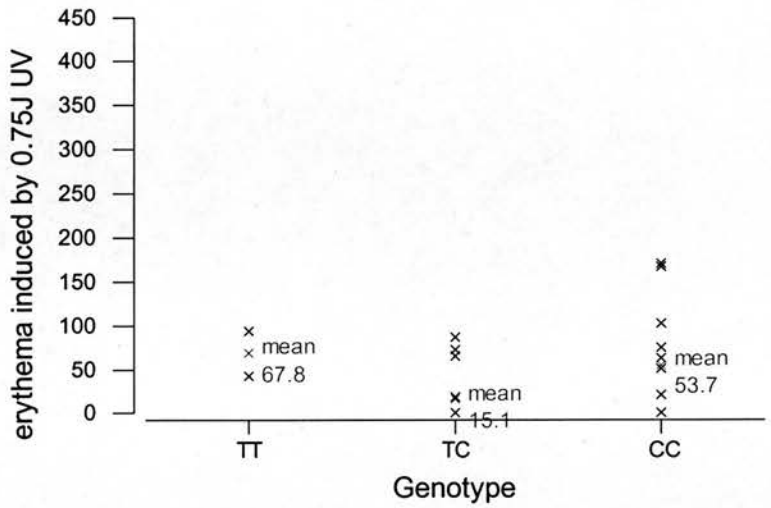


Figure 52. Erythema induced by 0.75J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

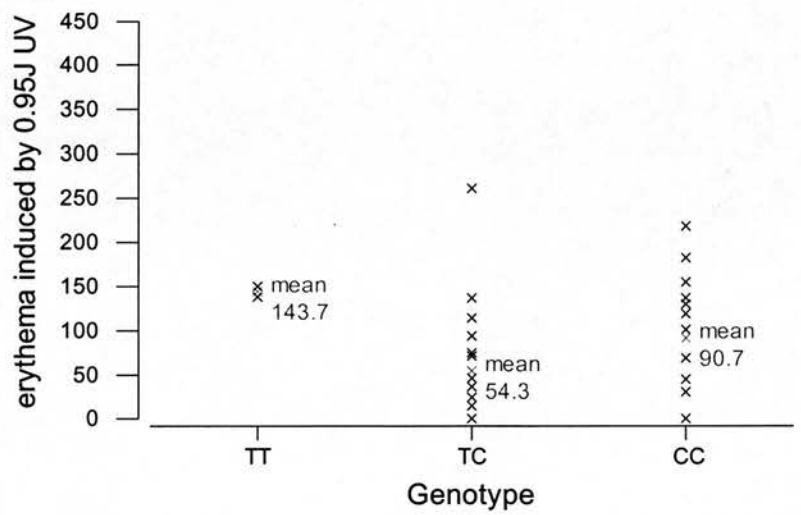


Figure 53. Erythema induced by 0.95J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

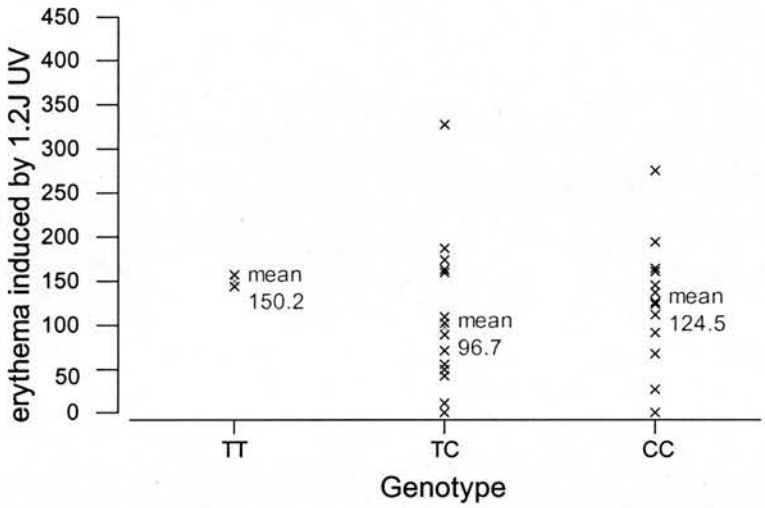


Figure 54. Erythema induced by 1.2J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

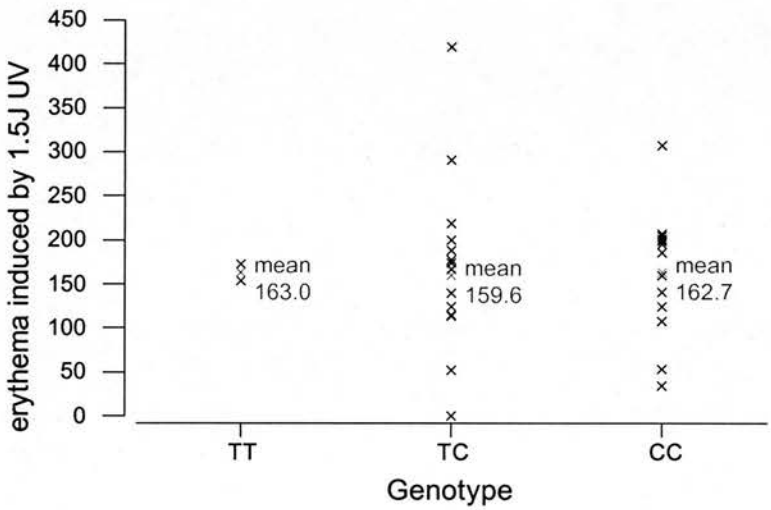


Figure 55. Erythema induced by 1.5J per cm^2 UV by CKM exon 8 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

Analysis of CKM exon 8 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	2.50	3.54	2.50
TC	16	0.125	0.50	0.125
CC	13	10.23	24.98	6.93

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	741	371	1.38	0.268
Error	28	7505	268		
Total	30	8246			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	0.389	1.243	0.293
CC	13	10.23	24.98	6.93

Source	DF	SS	MS	F	P
Factor	1	731	731	2.82	0.104
Error	29	7515	259		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	26.8	38.0	26.8
TC	16	3.88	10.81	2.70
CC	13	33.1	49.2	13.6

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6299	3149	2.73	0.082
Error	28	32257	1152		
Total	30	38555			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	6.43	15.59	3.67
CC	13	33.1	49.2	13.6

Source	DF	SS	MS	F	P
Factor	1	5361	5361	4.68	0.039
Error	29	33194	1145		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	67.8	30.6	21.7
TC	16	15.06	30.00	7.50
CC	13	53.7	60.9	16.9

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	23578	11789	5.60	0.009
Error	28	58956	2106		
Total	30	82535			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	26.2	43.6	10.3
CC	13	53.7	60.9	16.7

Source	DF	SS	MS	F	P
Factor	1	5704	5704	2.15	0.153
Error	29	76831	2649		
Total	30	82535			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	143.67	8.96	6.33
TC	16	54.3	71.1	17.8
CC	13	90.7	73.0	20.3

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	19451	9725	1.95	0.162
Error	28	139898	4996		
Total	30	159349			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	64.3	72.8	17.2
CC	13	90.7	73.0	20.3

Source	DF	SS	MS	F	P
Factor	1	5264	5264	0.99	0.328
Error	29	154085	5313		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	150.17	9.67	6.83
TC	16	96.8	88.4	22.1
CC	13	124.5	71.1	19.7

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8669	4334	0.68	0.514
Error	28	177919	6354		
Total	30	186588			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	102.7	84.8	20.0
CC	13	124.5	71.1	19.7

Source	DF	SS	MS	F	P
Factor	1	3597	3597	0.57	0.456
Error	29	182991	6310		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	2	163.0	14.1	10.0
TC	16	159.6	103.2	25.8
CC	13	162.7	72.2	20.0

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	73	36	0.00	0.995
Error	28	222369	7942		
Total	30	222442			

Comparison of TT and TC genotypes together with CC homozygotes:

Genotype	N	Mean	StDev	SE Mean
TT/TC	18	160.0	97.0	22.9
CC	13	162.7	72.2	20.0

Source	DF	SS	MS	F	P
Factor	1	53	53	0.01	0.934
Error	29	222389	7669		
Total	30	222442			

In group 2, no significant association was observed between any genotype and susceptibility to UVR at any dose examined when comparing the three genotypes. At 0.75J per cm^2 the mean level of erythema of the TC genotype did seem to be lower than that observed in TT and CC genotypes, however, this was not formally significant (P=0.06).

From the SS values, it can be seen that the CKM exon 8 genotype contributed little to the total amount of variation of the different levels of erythema observed. The SS(factor)

levels were extremely small, with the variation due to factors other than genotype (SS(error)) comprising almost all of the SS(total) values, for example at 1.5J per cm^2 the SS(total) value was 222442, with the genotype (SS(factor)) value being only 53.

In group 2, power calculations were performed using n=10 (balanced) and n=2 (lowest group size seen). At 0.47J, the between variance of 371 and within variance of 268 gave a power of 0.99 when n=10, and 0.24 when n=2. At 1.5J, the between variance was 36, and within variance 7942, giving powers of 0.06 when n=10 and 0.05 when n=2. These are extremely low, and are probably due to the small sample size, highlighting the need for greater numbers.

No association was observed between the CKM exon 8 genotype and erythemal response to UVR at any UV dose examined in either study group. CKM is located 3' to XPD on chromosome 19. If an association had been observed between XPD and erythemal response, this association may have extended to CKM. No such association was observed with respect to XPD, therefore the finding of no association between CKM and erythemal response was expected, as the gene has no (known) role in any of the DNA repair pathways.

ERCC1 exon 4

Along with XPF, ERCC1 acts as a 5' endonuclease, incising damaged DNA during NER. As NER repairs UV-induced damage, it is possible that if any polymorphisms of ERCC1 affect NER capability, this will reflect upon UVR sensitivity.

The ERCC1 exon 4 polymorphism is located at position 19007 on the GenBank entry M63796. The polymorphism was genotyped by a PCR-RFLP assay. PCR was used to generate a 252 base pair product. The polymorphism is a G to A change, which does not result in an amino acid substitution. The A allele contains a BsrDI restriction site, which gives two fragments to 179 and 73 (not seen on gel below) bp after digestion. The G allele does not contain this restriction site, and is therefore not cut, giving one fragment of 252 bp.

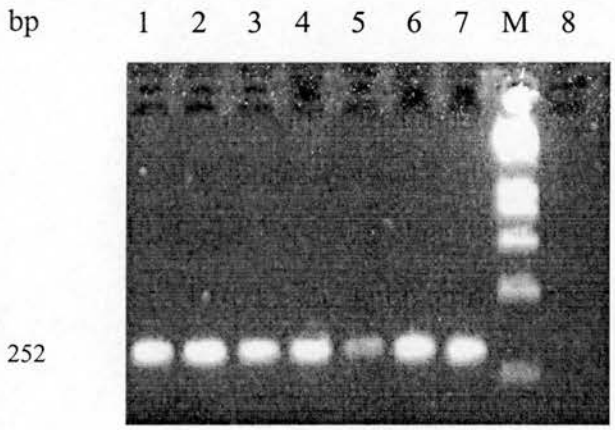


Figure 56. ERCC1 PCR products

Lanes 1-7, PCR product
Lane 8, negative control

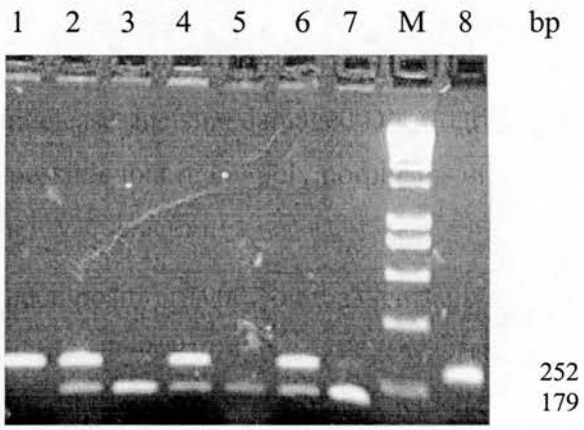


Figure 57 ERCC1 restriction digests

Lane 1, GG
Lanes 2,4,6, AG
Lanes 3, 5, 7, AA
Lane 8, uncut PCR product

ERCC1 exon 4 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
GG	13/74 (17.57%)	7/31 (22.58%)
GA	12/74 (16.21%)	7/31 (22.58%)
AA	49/74 (66.22%)	17/31 (54.84%)
Total (100%)	74 (100%)	31 (100%)

The AA homozygous genotype was most common in both study groups, accounting for the majority of individuals genotyped. The GG and GA genotype frequencies were similar to each other in both groups. No significant difference existed between the genotype frequencies of the two groups (χ^2 DF = 2, P-Value = 0.541).

Erythema induced by incremental doses of UVR by ERCC1 exon 4 genotype in group 1.

UVR on lower back, measured at 48 hours, n=74

Group 1

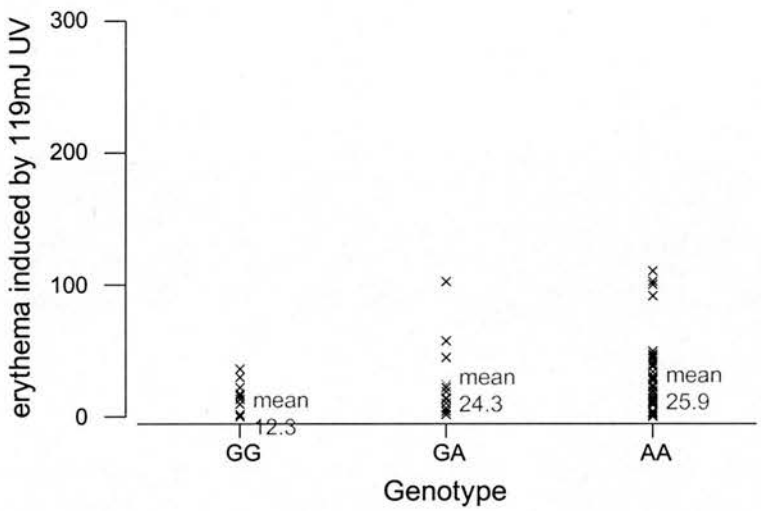


Figure 58. Erythema induced by 119mJ per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

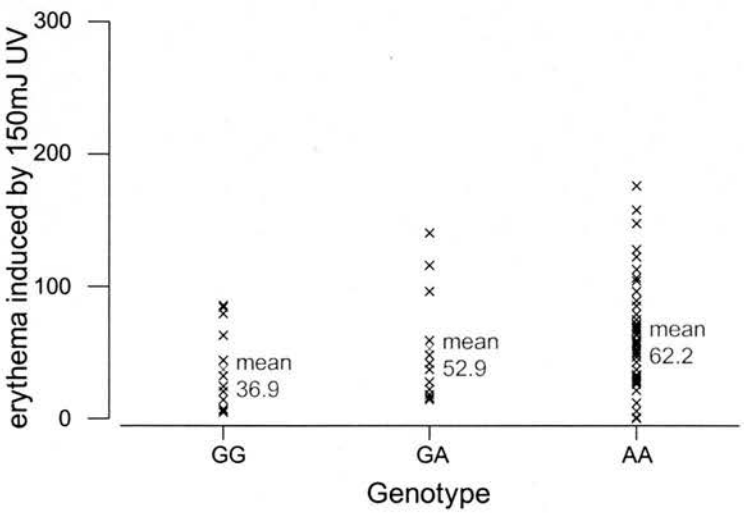


Figure 59 Erythema induced by 150mJ per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

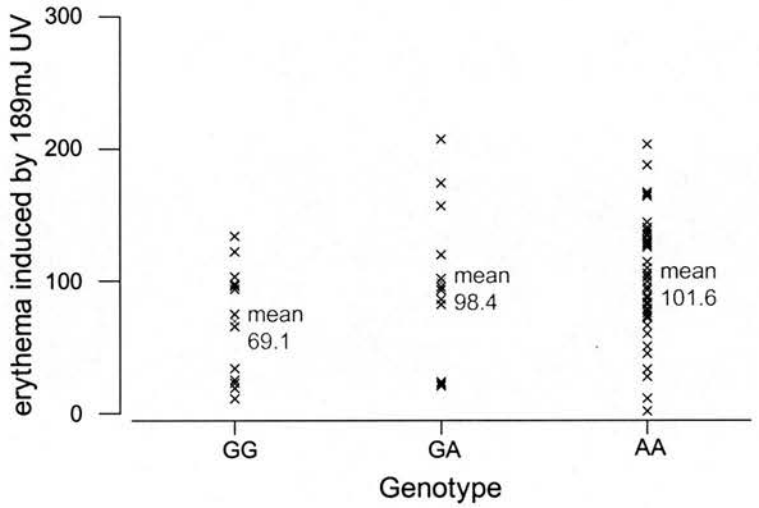


Figure 60 Erythema induced by 189mJ per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

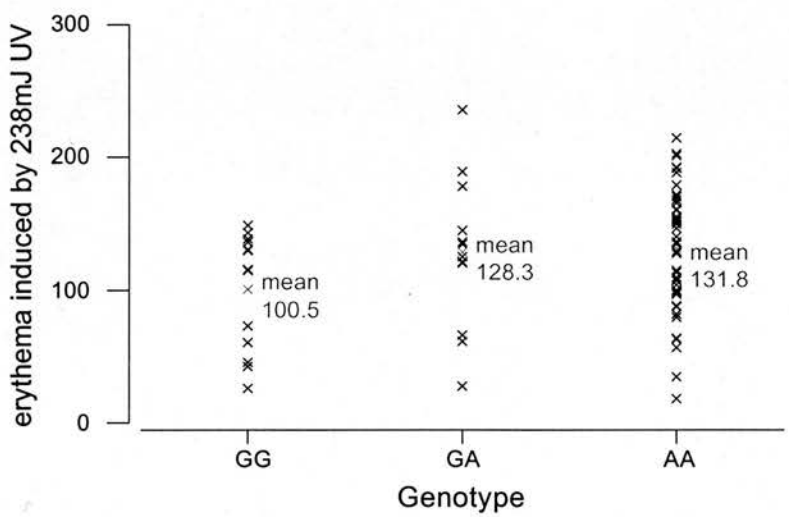


Figure 61. Erythema induced by 238mJ per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

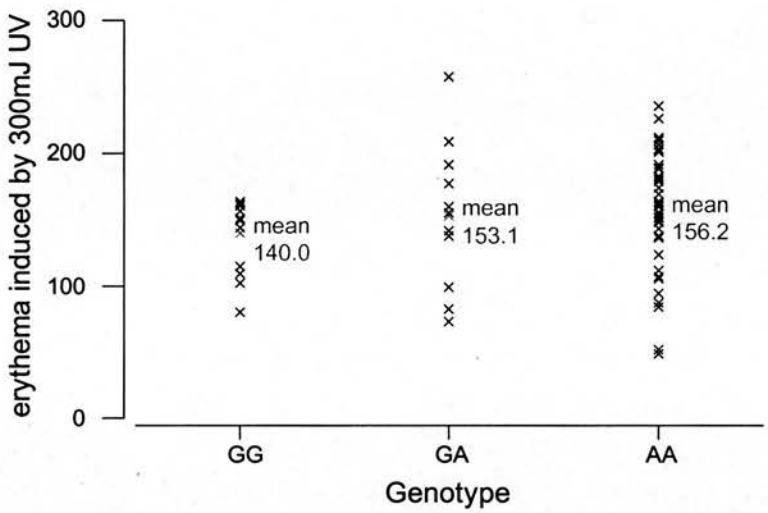


Figure 62. Erythema induced by 300mJ per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

Analysis of ERCC1 exon 4 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	13	12.26	12.42	3.45
GA	12	24.33	30.17	8.71
AA	49	25.91	26.64	3.81

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1931	966	1.49	0.232
Error	71	45930	647		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	13	36.91	30.96	8.59
GA	12	52.9	42.2	12.2
AA	49	62.20	39.41	5.63

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6729	3365	2.26	0.112
Error	71	105652	1488		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	13	69.1	42.4	11.7
GA	12	98.4	59.8	17.3
AA	49	101.58	44.34	6.33

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	10996	5498	2.51	0.088
Error	71	155322	2188		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	13	100.5	44.1	12.2
GA	12	128.3	57.7	16.7
AA	49	131.77	44.48	6.35

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	10139	5069	2.32	0.105
Error	71	154956	2182		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	13	140.01	28.24	7.83
GA	12	153.1	52.8	15.2
AA	49	156.18	42.48	6.07

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2688	1344	0.75	0.475
Error	71	126795	1786		
Total	73	129483			

In group 1, the AA genotype displayed higher mean levels of erythema at each UV dose examined. The GG genotype similarly displayed the lowest levels of erythema, with the heterozygotes falling between the two. This was not significant at any dose examined, although at 189mJ per cm^2, the level of significance was approached ($p=0.08$). Although the level of significance was approached at 189mJ per cm^2, a comparison of factor and error reveal that the genotype explains very little of the total variation between erythema levels, SS(factor) being 10996, while SS(error) equals 155322, and a SS(total) value of 166318. As p values are not significant at any other UV dose examined, and, from factor and error values, it can be determined that most of the variation in erythemal response is

due to factors other than genotype, the ERCC1 exon 4 polymorphism is not implicated in the erythemal response to UV in group 1.

Power calculations were carried out in group 1 using n=25 (balanced ANOVA) and n=12 (most unbalanced) to give a range of true values the power would lie within. At 119mJ UV, the between variance value of 966 and within variance value of 647 gave a power of 1 when n=25 and a power of 0.99 when n=12. At 300mJ, between variance was 1344, and within variance 1786, which, when n=25 gave a power of 0.99 and when n=12, a power of 0.96. For this polymorphism the sample size in group 1 appears to be adequate due to the acceptable level of power. It can be confidently said that the ANOVA results which indicated that the ERCC1 polymorphism is not associated with erythemal response is likely to be true.

Erythema induced by incremental doses of UVR by ERCC1 exon 4 genotype in group 2.

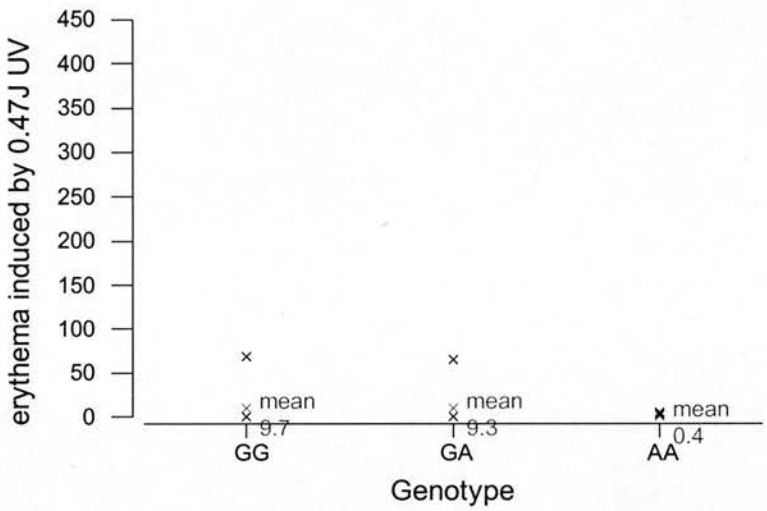


Figure 63 Erythema induced by 0.47J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

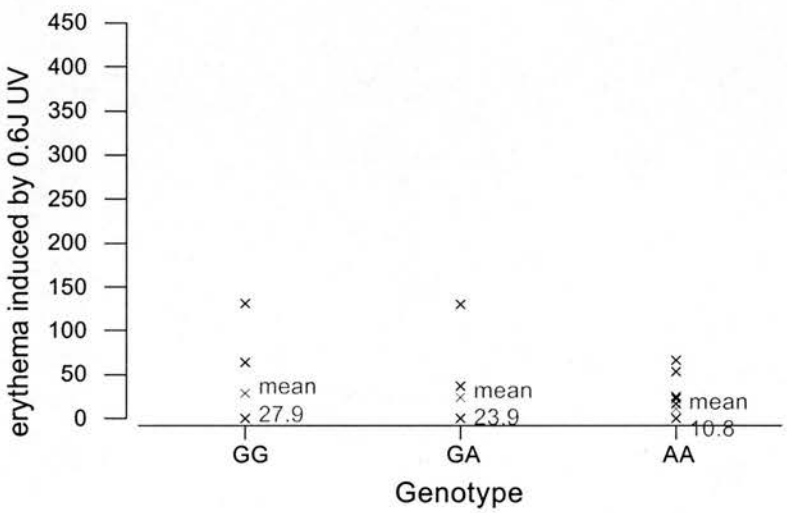


Figure 64. Erythema induced by 0.6J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

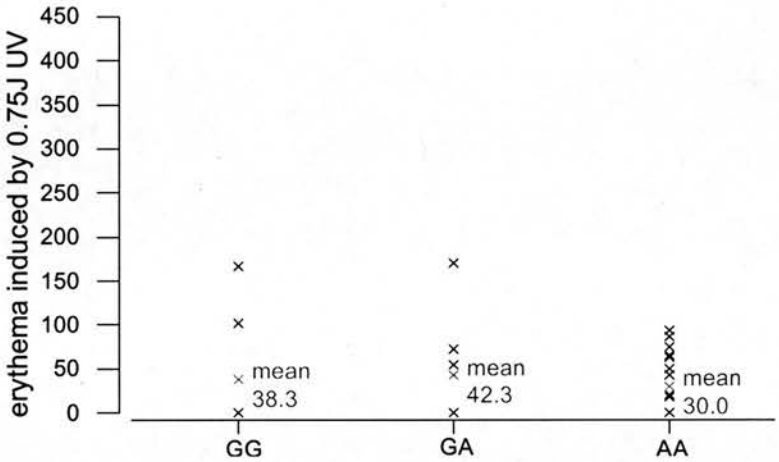


Figure 65 Erythema induced by 0.75J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

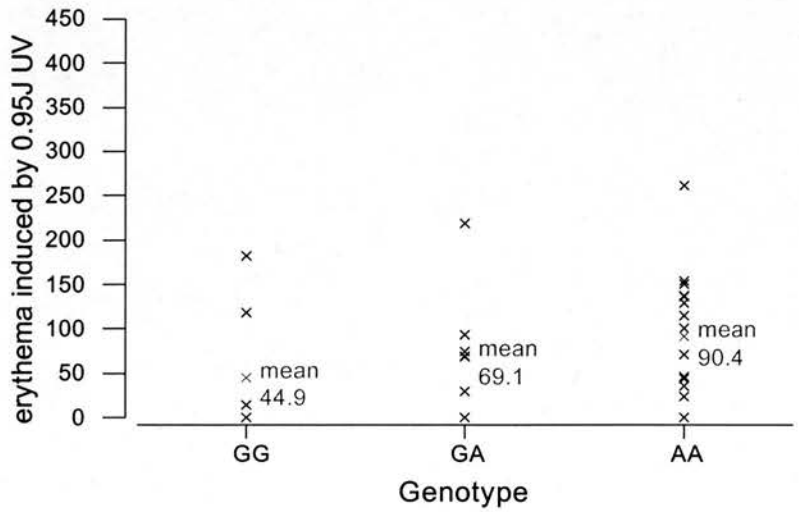


Figure 66. Erythema induced by 0.95J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

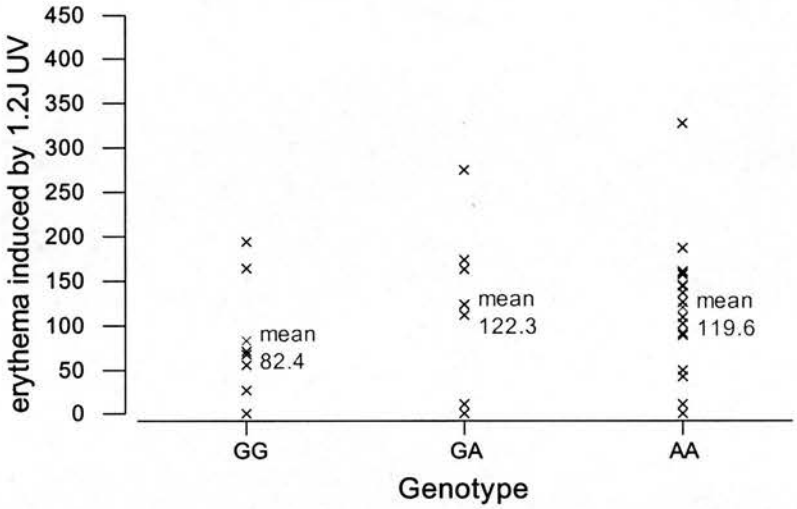


Figure 67 Erythema induced by 1.2J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

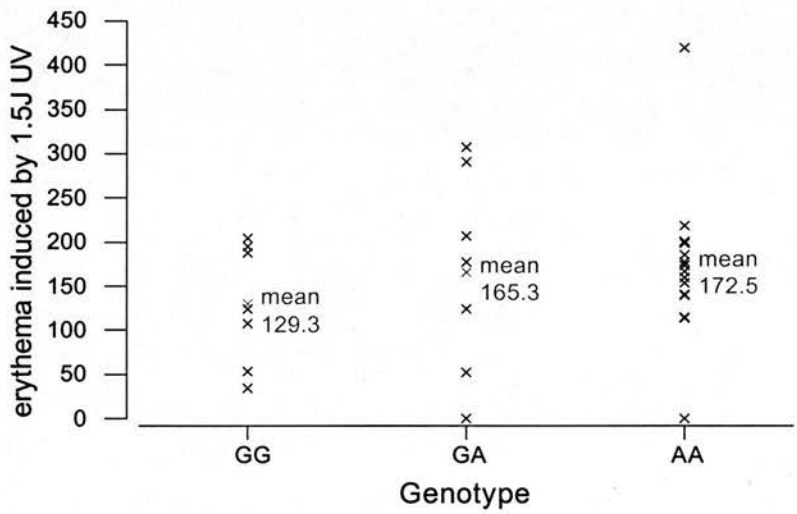


Figure 68 Erythema induced by 1.5J per cm^2 UV by ERCC1 exon 4 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

Analysis of ERCC1 exon 4 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	9.71	25.70	9.71
GA	7	9.29	24.57	9.29
AA	17	0.412	1.278	0.310

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	635	317	1.17	0.326
Error	28	7611	272		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	27.9	51.4	19.4
GA	7	23.9	48.8	18.4
AA	17	10.80	20.39	4.94

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1796	898	0.68	0.513
Error	28	36760	1313		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	38.3	67.9	25.7
GA	7	42.3	63.9	24.1
AA	17	30.04	34.79	8.44

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	862	431	0.17	0.846
Error	28	71546	2555		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	44.9	74.3	28.1
GA	7	69.1	75.2	28.4
AA	17	90.5	71.4	17.3

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	10659	5330	1.00	0.379
Error	28	148690	5310		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	82.4	70.9	26.8
GA	7	122.3	95.8	36.2
AA	17	119.6	76.5	18.5

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7857	3928	0.62	0.548
Error	28	178731	6383		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	7	129.3	69.3	26.2
GA	7	165.3	115.2	43.5
AA	17	172.5	80.9	19.6

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	9395	4698	0.62	0.547
Error	28	213047	7609		
Total	30	222442			

At the highest three doses examined in group 2, a similar pattern to that seen in group 1 was observed, with the AA genotype having higher mean levels of erythema than the heterozygotes, which in turn had higher mean erythema levels than the GG genotype. This was not significant, and P values ranged from 0.846 to 0.326, indicating that in group 2, similar to group 1, the ERCC1 exon 4 polymorphism is not associated with UV-induced erythema.

Power calculations were performed for group 2 using n=10 (balanced) and n=7 (unbalanced). At 0.47J, the between variance was 317 and within variance 272. When n=10 this gave a power of 0.99 and 0.92 when n=7. At 1.5J, the between variance was 4698 and the within variance 7609, giving a power of 0.85 when n=10, and 0.67 when n=7. For this polymorphism the levels of power are acceptable, and the hypothesis that ERCC1 exon 4 genotype is associated with erythemal response can be rejected due to non-significant p values in the analysis of variance with confidence.

In their study of melanoma risk, Winsey and colleagues (2000) analysed the ERCC1 exon 4 polymorphism. No association was observed between melanoma risk and exon 4 genotype in 125 patients and 211 controls. However, as mentioned above, the controls in this study were cadavers. In their report, Winsey and colleagues do not discuss whether the controls had any past history of skin cancer, which could have had an effect on the statistical analysis. However, if one were to assume no history of melanoma in the cadaver group, this relatively large study is in agreement with that observed by this research project. No association was observed between the ERCC1 exon 4 polymorphism and erythemal response, a measure of cutaneous sensitivity to UVR. This implies this polymorphism is not associated with a decreased NER capacity, in the repair of UV-induced DNA photoproducts, which is reinforced by the study by Winsey and colleagues, which found no association between this polymorphism and melanoma.

XPF exon 11

As a component, along with ERCC1, of the endonuclease that incises DNA 5' to damage during NER, it is feasible that polymorphisms in the XPF gene might affect ability of NER to repair UV-induced DNA damage, and susceptibility to UVR.

To genotype for XPF exon 11, a PCR product of 709 bp was produced, which contains the polymorphism at exon 11. This polymorphism consists of a T to C change, at position 30028 on GenBank entry L76568. The T allele contains an AlwNI restriction site, which results in two fragments, of 442 and 267 bp. The C allele has no AlwNI restriction site, and yields a single fragment of 709 bp.

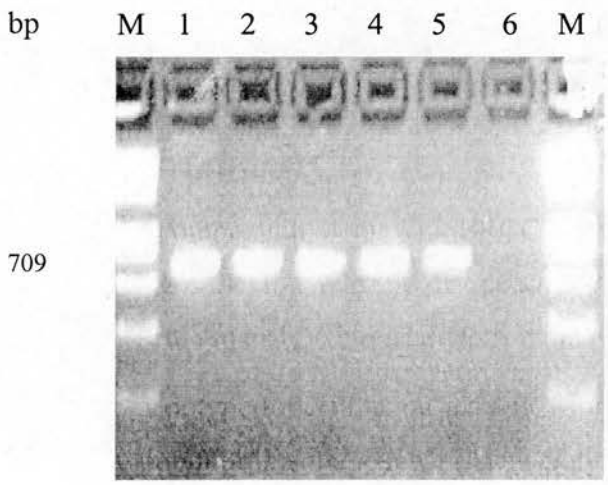


Figure 69, XPF exon 11 PCR products

Lanes 1-5, PCR products
Lane 6, negative control

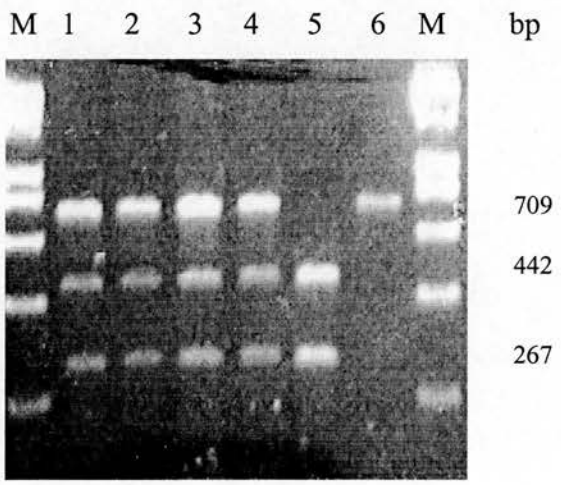


Figure 70, XPF exon 11 digest

Lanes 1-4, TC
Lane 5, TT
Lane 6, CC

XPF exon 11 Genotype frequencies

Genotype	Group 1(%)	Group 2 (%)
TT	34/74 (45.95%)	18/31 (58.06%)
TC	33/74 (44.59%)	10/31 (32.26%)
CC	7/74 (9.46%)	3/31 (9.68%)
Total (100%)	74 (100%)	31 (100%)

The CC genotype frequency was extremely similar in the two study groups, although there were slight differences in the frequencies of the TT and TC genotypes within the two study groups. The genotype frequencies observed were not statistically different from each other (χ^2 DF = 2, P-Value = 0.482)

Erythema induced by incremental doses of UVR by XPF exon 11 genotype in group 1.

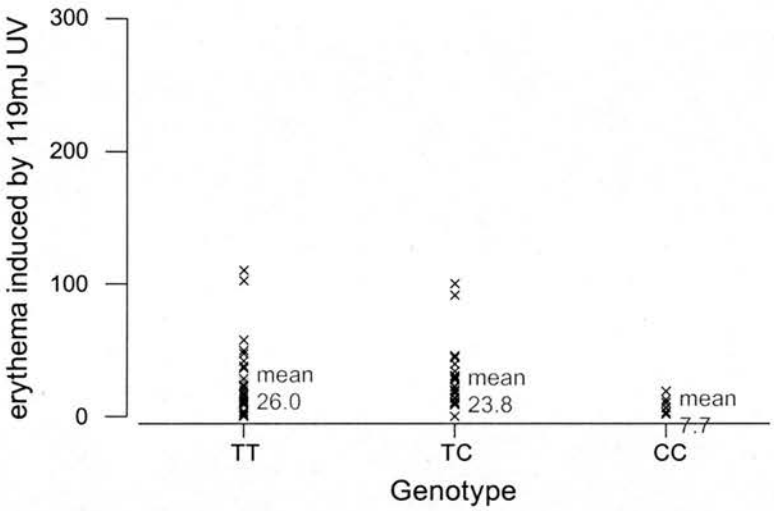


Figure 71 Erythema induced by 119mJ per cm^2 UV by XPF exon 11 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

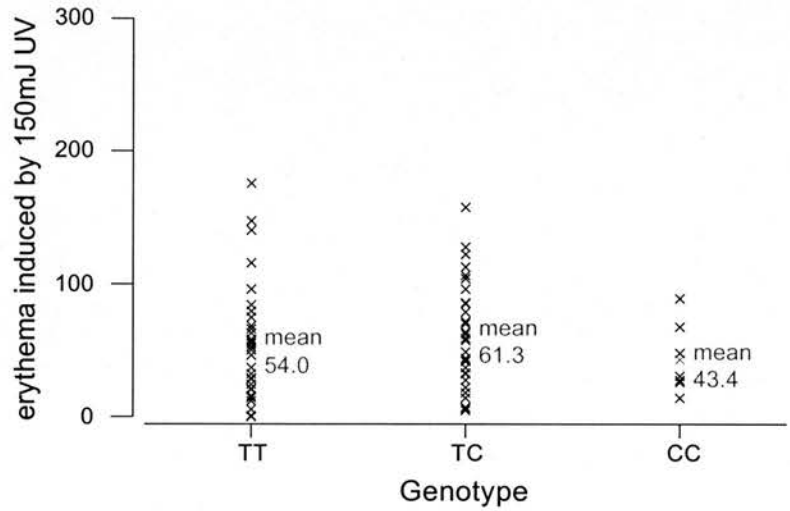


Figure 72 Erythema induced by 150mJ per cm^2 UV by XPF exon 11 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

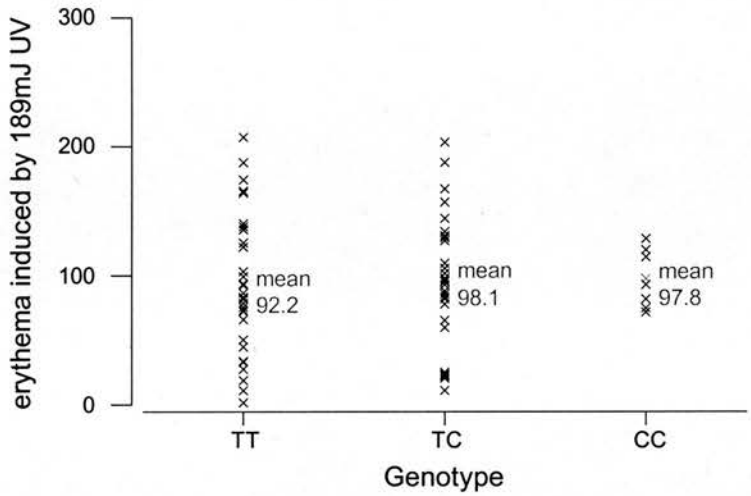


Figure 73 Erythema induced by 189mJ per cm^2 UV by XPF exon 11 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

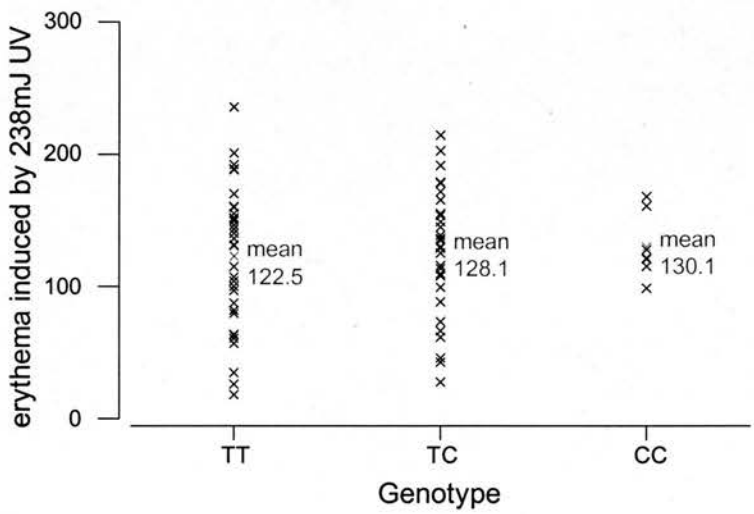


Figure 74 Erythema induced by 238mJ per cm^2 UV by XPF exon 11 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

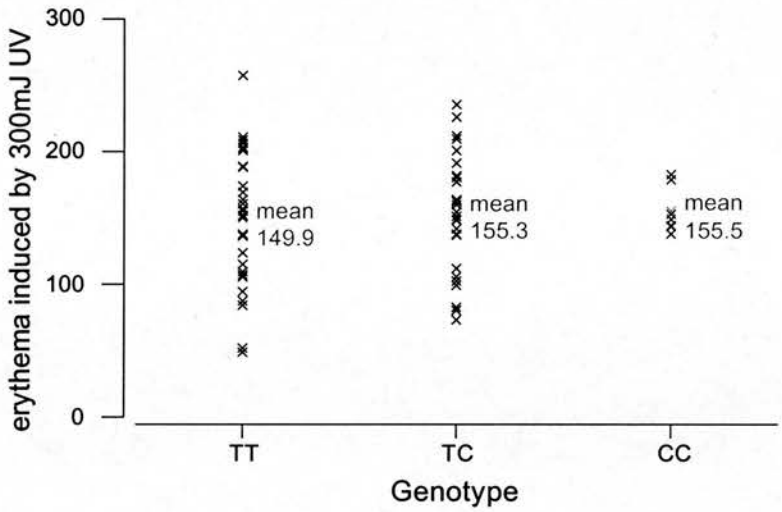


Figure 75. Erythema induced by 300mJ per cm^2 UV by XPF exon 11 genotype.
UVR induced on lower back, measured at 48 hours, n=74.

Analysis of XPF exon 11 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	34	25.96	29.59	5.07
TC	33	23.76	22.90	3.99
CC	7	7.74	6.39	2.42

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1943	972	1.50	0.230
Error	71	45918	647		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	34	53.97	42.61	7.31
TC	33	61.33	37.94	6.60
CC	7	43.4	26.5	10.0

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2183	1091	0.70	0.498
Error	71	110199	1552		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	34	92.20	50.94	8.74
TC	33	98.10	49.02	8.53
CC	7	97.81	22.94	8.67

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	630	315	0.13	0.874
Error	71	165688	2334		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	34	122.47	52.94	9.08
TC	33	128.13	46.16	8.04
CC	7	130.14	24.99	9.44

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	688	344	0.15	0.862
Error	71	164406	2316		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	34	149.88	47.24	8.10
TC	33	155.33	40.84	7.11
CC	7	155.50	17.92	6.77

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	552	276	0.15	0.859
Error	71	128930	1816		
Total	73	129483			

In group 1, no pattern was seen in response to UV, and this was confirmed by statistical analysis. No statistically significant association was observed at any of the UV doses examined. P values increased as the UV doses examined increased, p=0.230 for 119mJ per cm^2, p=0.859 for 300mJ per cm^2. A comparison of factor and error reveals that most of the variation observed in erythemal response is due to external factors other than the XPF exon 11 polymorphism.

In group 1, the balanced ANOVA would have had all three groups contained 25 individuals. The least frequent genotype had 7 individuals in it, therefore n=7 was used as

the least balanced option for the power calculations. At 119mJ, the between variance value was 972, and the within variance value was 647. This gave a power of 1 where n=25, and 0.97 when n=7. The between variance was 276 at 300mJ, and the within variance 1816, giving power of 0.67 when n=25 and 0.21 when n=7. The difference in power at the two UV doses is probably due to more variation at the higher dose. Increased power, which could be achieved by increasing the sample size, could allow greater accuracy of the statistical analysis.

Erythema induced by incremental doses of UVR by XPF exon 11 genotype in group 2.

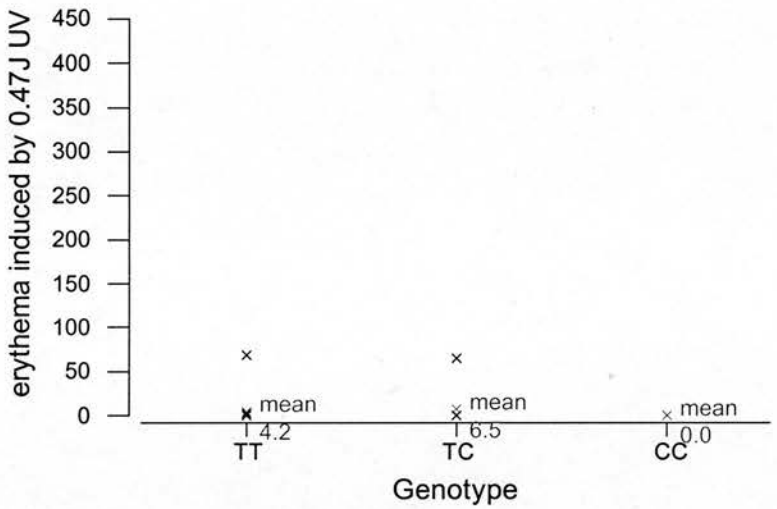


Figure 76. Erythema induced by 0.47J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

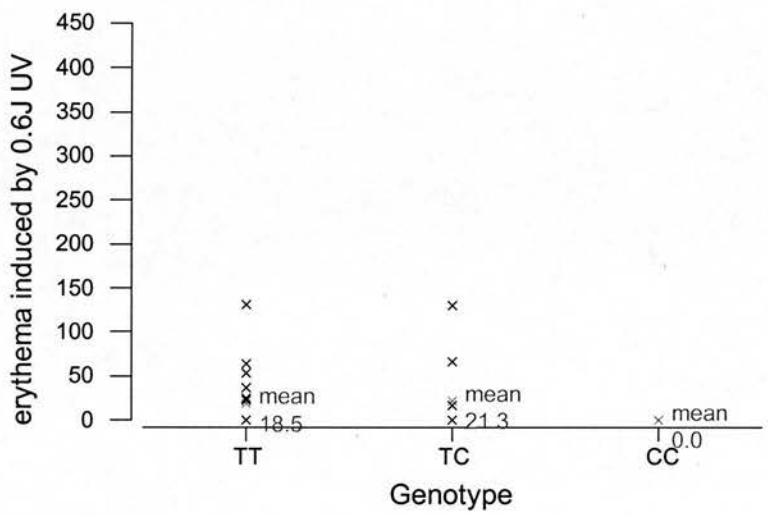


Figure 77. Erythema induced by 0.6J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

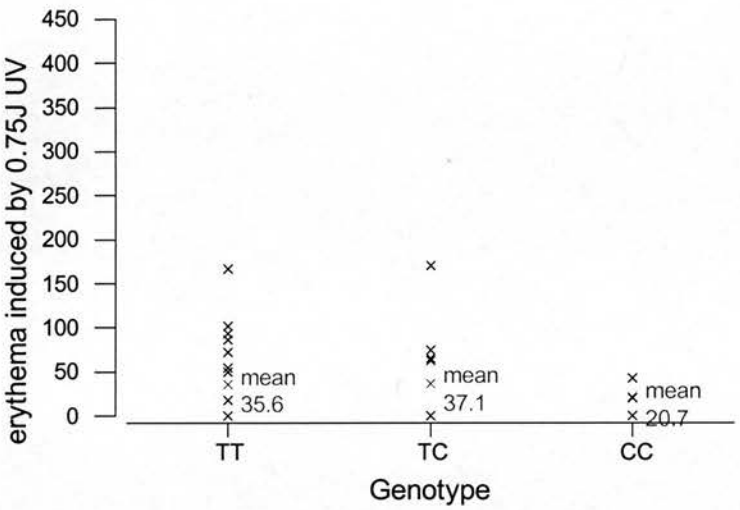


Figure 78 Erythema induced by 0.75J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

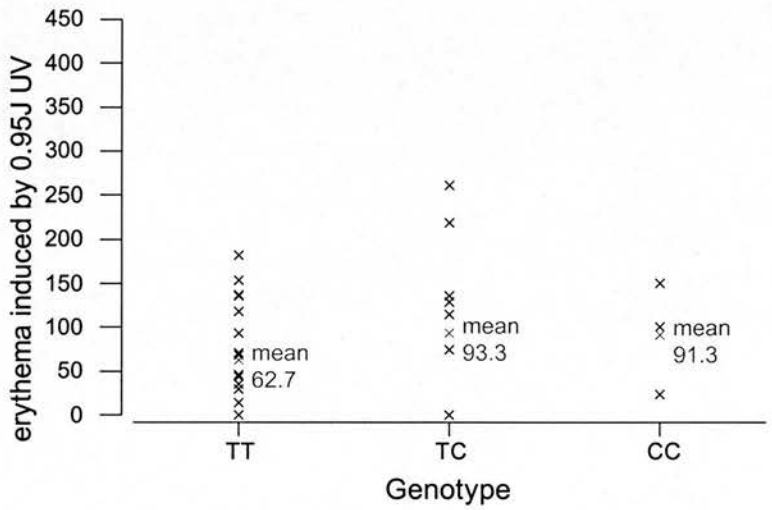


Figure 79 Erythema induced by 0.95J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

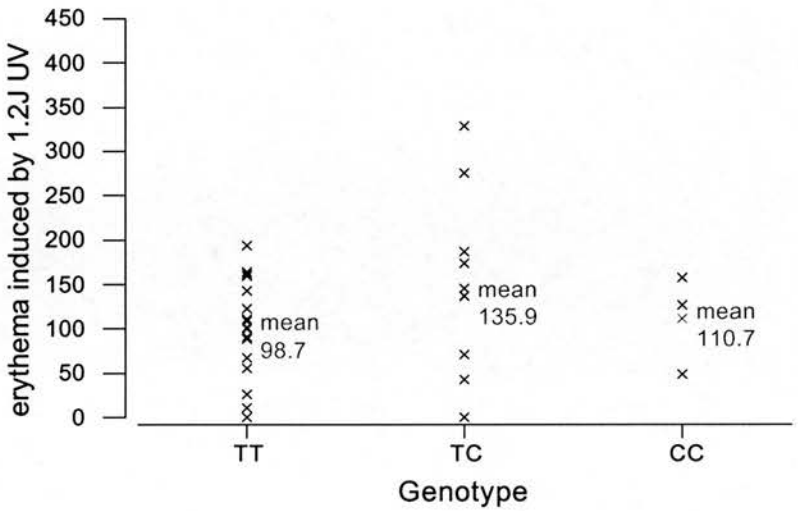


Figure 80 Erythema induced by 1.2J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

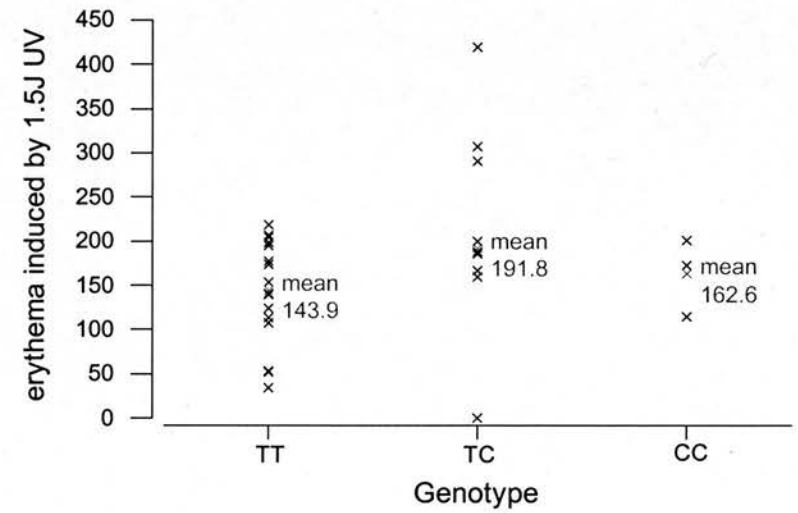


Figure 81 Erythema induced by 1.5J per cm^2 UV by XPF exon 11 genotype.
UVR induced on inner forearm, measured at 24 hours, n=31.

Analysis of XPF exon 11 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	4.17	15.98	3.77
TC	10	6.50	20.55	6.50
CC	3	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	103	51	0.18	0.839
Error	28	8143	291		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	18.48	34.70	8.18
TC	10	21.3	43.5	13.7
CC	3	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1080	540	0.40	0.672
Error	28	37475	1338		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	35.6	49.8	11.7
TC	10	37.1	56.5	17.9
CC	3	20.7	21.0	12.1

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	665	332	0.13	0.879
Error	28	71743	2562		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	62.7	60.4	14.2
TC	10	93.3	95.7	30.3
CC	3	91.3	63.4	36.6

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6855	3428	0.63	0.540
Error	28	152494	5446		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	98.7	60.0	14.1
TC	10	135.9	110.7	35.0
CC	3	110.7	55.6	32.1

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8917	4458	0.70	0.504
Error	28	177671	6345		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
TT	18	143.9	56.2	13.2
TC	10	191.8	129.1	40.8
CC	3	162.6	44.3	25.6

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14753	7377	0.99	0.383
Error	28	207689	7417		
Total	30	222442			

In group 2, the TC genotype had the highest mean values of level of erythema at each UV dose, and TT the lowest, except at 0.75J per cm^2. This was not significant, with wide errors. P values ranged from 0.879 to 0.383, and erythemal response varied between individuals due to other factors than genotype, as can be seen by low SS(factor) values compared with SS(error) and SS(total) values. No association can be claimed on the basis of these results between XPF exon 11 and UV sensitivity.

N=10 and n=3 were used in the power calculations for group 2 to give a range for power to fall within. 0.47J gave a between variance value of 51, and a within variance value of 1291, which gave a power of 0.11 when n=10, and 0.06 when n=3. 1.5J gave a between variance value of 7377 and a within variance value of 7417, resulting in a power of 0.97 when n=10 and 0.38 when n=3. The power of the lower dose is too low to draw any conclusive result from, and suggests the higher UV doses are likely to have a higher power, but greater power at all doses would be ideal.

XPG exon 15

The polymorphism at exon 15 of XPG results in a non-synonymous amino acid change, from His to Asp. The polymorphism is a G to C change, which is present at position 3507 on the cDNA of GenBank entry NM_000123. A 271 bp PCR product was generated, which was digested with NlaIII in order to genotype samples. The C allele contains a NlaIII restriction site, and yields two fragments of 227 and 44 bp (44bp fragment not seen on gel below). The G allele does not have a restriction site for NlaIII, and is uncut by the enzyme, giving a single fragment of 271 bp. The heterozygous CG genotype yields fragments of 271, 227 and 44 bp.

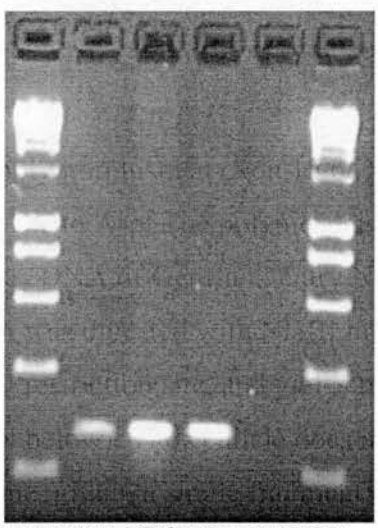


Figure 82 XPG exon 15 PCR

Lanes 1-3 PCR products

Lane 4 negative control

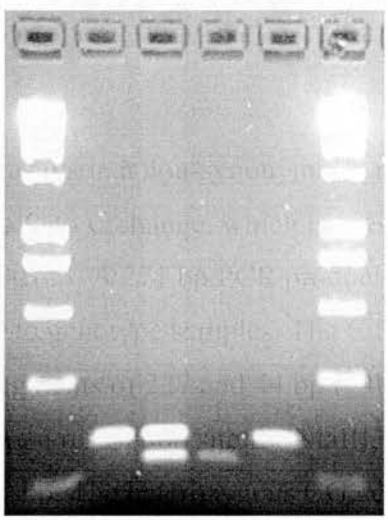


Figure 83 XPG exon 15 digest

Lane 1 GG

Lane 2 CG
Lane 3 CC
Lane 4 undigested PCR product

XPG exon 15 Genotype frequencies

The XPG exon 15 shows global variation in the frequency of the different genotypes. The CC genotype is rare in Caucasians, but more frequent in Asians and Africans, where the majority of the population are heterozygous for the polymorphism.

Genotype frequencies in different populations:

Genotype	Scottish healthy N=277	Ivory Coast samples N=30	Korean healthy controls N=195
CC	3.6%	26.67%	31.3%
CG	35%	61.4%	46.7%
GG	61.4%	16.67%	22%
Total	100%	100%	100%

(Scottish healthy sample data, personal communication with Prof David Melton, Korean healthy control data, Jeon *et al*, 2003. Ivory Coast samples were a kind donation from Dr Rosalind Harding, Oxford University, and were genotyped as above alongside samples from Group 1 and 2).

The allele frequencies of the exon 15 polymorphism showed considerable variation between the different populations, in particular between the Scottish and African groups. In the Scottish population, the homozygous CC genotype accounted for only 3.6% of the total, and the heterozygous CG 38%, whilst the Ivory Coast samples had genotype frequencies of 26.67% for the CC genotype, and 61.4% for the CG genotype. This difference in genotype frequencies could be due to genetic drift caused by a Founder effect, where changes in the gene pool of a particular population are attributable to colonisation by a limited number of individuals from a parent population and could have no survival advantage. Alternatively, the CC genotype frequency in the Ivory Coast

samples could be a result of natural selection, where having the C allele conferred a degree of evolutionary advantage in that population, for example, if the XPG exon 15 genotype is associated with cutaneous sensitivity to UVR, it could be that the CC genotype confers greater protection against UVR, which would be an advantage in Africa due to the increased exposure to UVR of the local population. This would be much less of an advantage in Scotland, however, where exposure to UVR is greatly reduced. It is also possible that the XPG exon 15 polymorphism frequency variation between populations is due to a confounding association with another gene, which itself is present in the population as a result of a Founder effect or a selection advantage.

Genotype frequencies in this study:

Genotype	Group 1(%)	Group 2 (%)
CC	4/74 (5.40%)	2/31 (6.45%)
CG	20/74 (27.03%)	6/31 (19.35%)
GG	50/74 (67.57%)	23/31 (74.20%)
Total	74 (100%)	31 (100%)

Genotype frequencies were similar in the two groups studies here (χ^2 DF = 2, P-Value = 0.705)

(As the frequency of CC homozygotes was extremely low (less than 7% in each group), as well as analysing variance between each genotype, the CC homozygotes were grouped with the CG heterozygotes for further analysis.

Erythema induced by incremental doses of UVR by XPG exon 15 genotype in group 1.

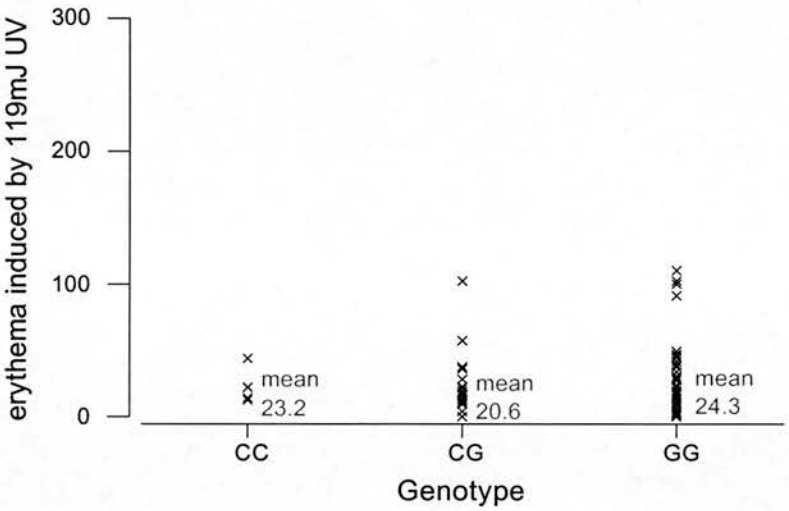


Figure 84 Erythema induced by 119mJ per cm^2 UV by XPG exon 15 genotype.
UVR on lower back, measured at 48 hours, n=74.

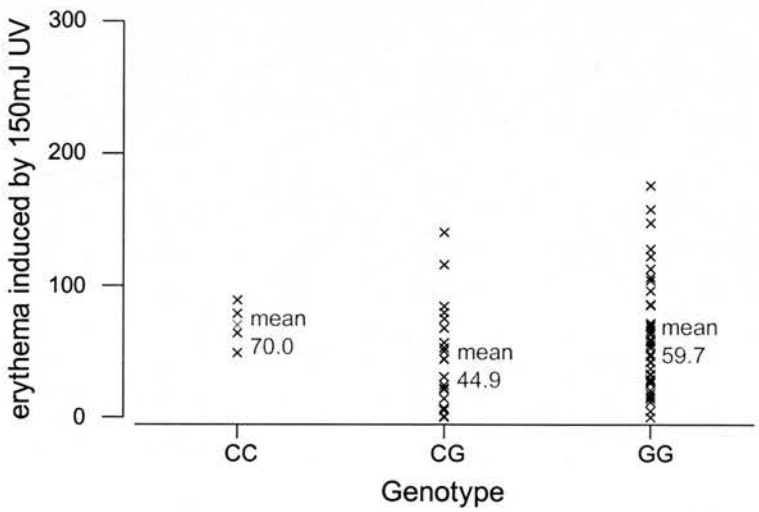


Figure 85 Erythema induced by 150mJ per cm^2 UV by XPG exon 15 genotype.
UVR on lower back, measured at 48 hours, n=74.

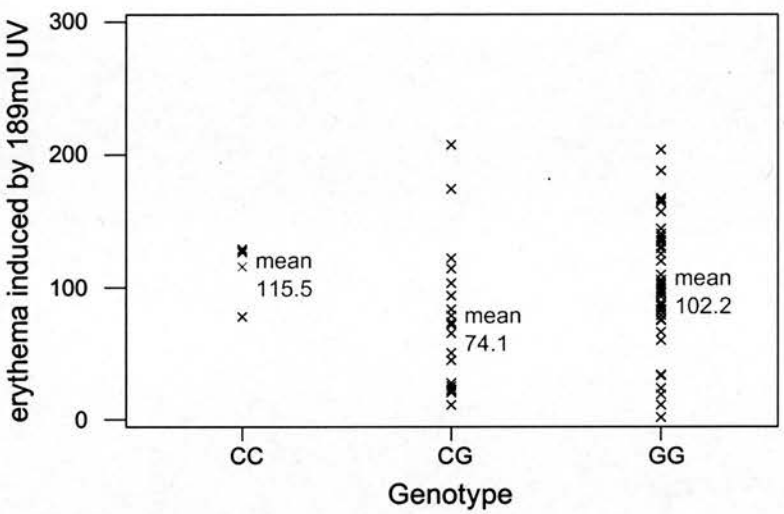


Figure 86 Erythema induced by 189mJ per cm^2 UV by XPG exon 15 genotype.
UVR on lower back, measured at 48 hours, n=74.

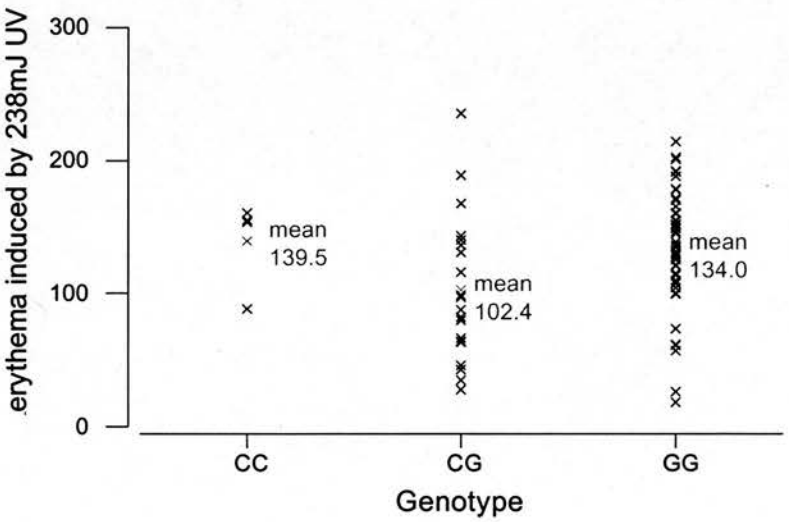


Figure 87 Erythema induced by 238mJ per cm^2 UV by XPG exon 15 genotype.
UVR on lower back, measured at 48 hours, n=74.

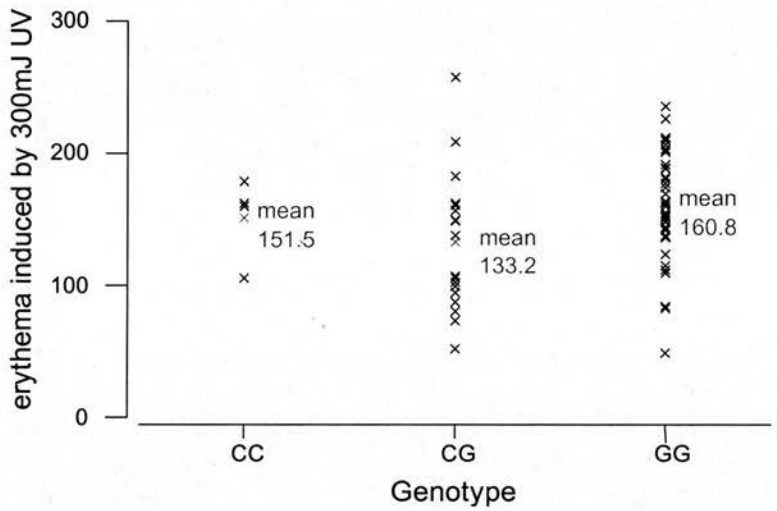


Figure 88 Erythema induced by 300mJ per cm^2 UV by XPG exon 15 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of XPG exon 15 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	4	23.17	14.72	7.36
CG	20	20.63	24.42	5.46
GG	50	24.32	26.99	3.82

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	195	97	0.14	0.865
Error	71	47666	671		
Total	73	47861			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	24	21.05	22.85	4.66
GG	50	24.32	26.99	3.82

Source	DF	SS	MS	F	P
Factor	1	173	173	0.26	0.611
Error	72	47688	662		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	4	70.00	17.64	8.82
CG	20	44.90	39.50	8.83
GG	50	59.69	39.87	5.64

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3925	1963	1.28	0.283
Error	71	108457	1528		
Total	73	112382			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	24	49.08	37.69	7.69
GG	50	59.69	39.87	5.64

Source	DF	SS	MS	F	P
Factor	1	1825	1825	1.19	0.279
Error	72	110557	1536		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	4	115.5	25.2	12.6
CG	20	74.1	51.9	11.6
GG	50	102.23	45.21	6.39

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	12987	6494	3.01	0.056
Error	71	153330	2160		
Total	73	166318			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	24	81.0	50.6	10.3
GG	50	102.23	45.21	6.39

Source	DF	SS	MS	F	P
Factor	1	7277	7277	3.29	0.074
Error	72	159041	2209		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	4	139.5	34.2	17.1
CG	20	102.4	54.9	12.3
GG	50	133.96	42.66	6.03

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	15072	7536	3.57	0.033
Error	71	150023	2113		
Total	73	165094			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	24	108.5	53.3	10.9
GG	50	133.96	42.66	6.03

Source	DF	SS	MS	F	P
Factor	1	10474	10474	4.88	0.030
Error	72	154621	2148		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	4	151.5	32.0	16.0
CG	20	133.2	49.8	11.1
GG	50	160.81	37.34	5.28

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	10914	5457	3.27	0.044
Error	71	118569	1670		
Total	73	129483			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	24	136.23	47.25	9.65
GG	50	160.81	37.34	5.28

Source	DF	SS	MS	F	P
Factor	1	9801	9801	5.90	0.018
Error	72	119682	1662		
Total	73	129483			

In group 1, the two lowest doses of UV showed no significant association between any genotype and level of erythema produced, either when comparing the three genotypes separately, or when combining the CC and CG genotypes, and comparing these to the GG genotype. At the higher UV doses examined, the CC and CG genotypes had significantly lower levels of erythema than the GG genotype ($p = 0.033$ at 238mJ per cm^2, $p = 0.044$ at 300mJ per cm^2).

In group 1, n=25 and n=4 were used to determine the power of the ANOVA tests. At 119mJ, the between variance of 97 and within variance of 671 gave a power of 0.65 when n=25, and 0.12 when n=4, the true power lying between these values. At 300mJ, the between variance was 5457 and within variance 1670, which gave a power of 1 when n=25 and a power of 0.97 when n=4. At the higher UV dose, the power is high enough to assume the p value of 0.044 is correct, and that there was a significant difference between genotypes. A larger study would increase the power, and perhaps increase the level of significance.

When the CC and CG genotypes were combined and compared together against the GG genotype, the level of significance increased to $p=0.03$ at 238mJ per cm^2 and $p=0.018$ at 300mJ per cm^2. Comparing factor with error revealed that, although at the highest doses there as significant association between genotype and erythemal response, factors other than genotype were responsible for much of the variation in erythemal values, for

example, at 300mJ per cm^2, where p=0.018 for combined CC/CG genotypes against the GG genotype, the SS(factor) value was 9801, whilst the SS(error) was 119682, contributing towards most of the SS(total) of 129483.

As this polymorphism results in an amino acid change at the protein level, it is possible that this association, with the GG genotype being more susceptible to UVR, might be causative.

Erythema induced by incremental doses of UVR by XPG exon 15 genotype in group 2.

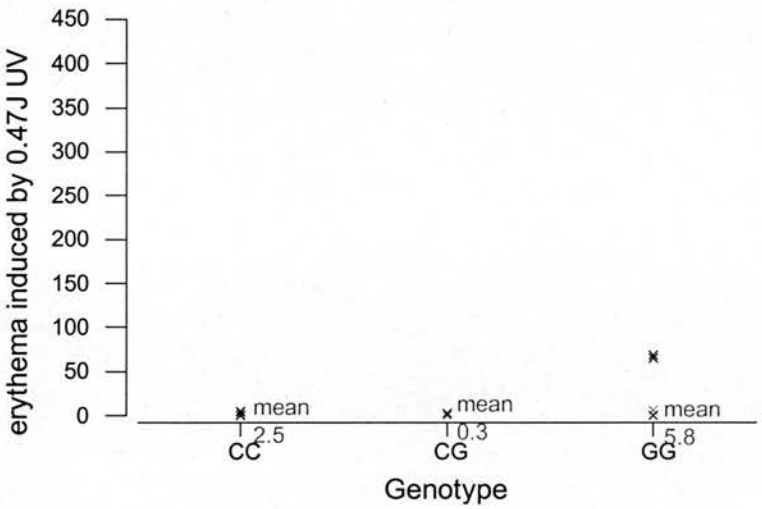


Figure 89.Erythema induced by 0.47J per cm^2 UV by XPG exon 15 genotype.
UV on inner forearm, measured at 24 hours

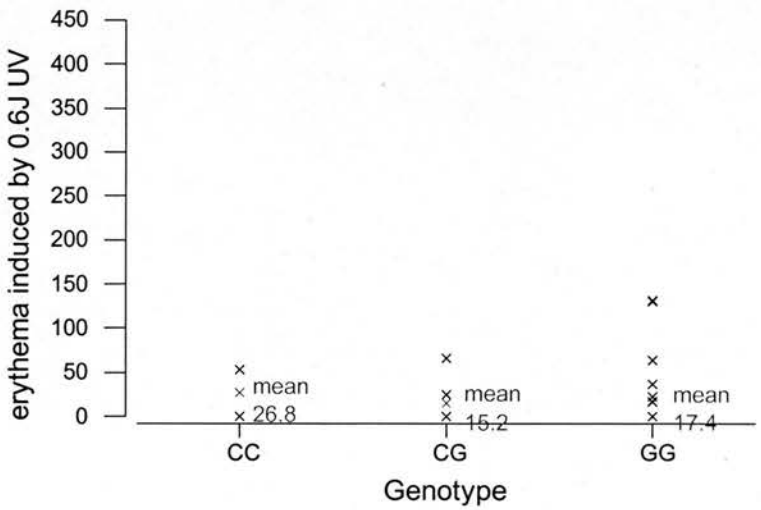


Figure 90 Erythema induced by 0.6J per cm^2 UV by XPG exon 15 genotype.
UV on inner forearm, measured at 24 hours

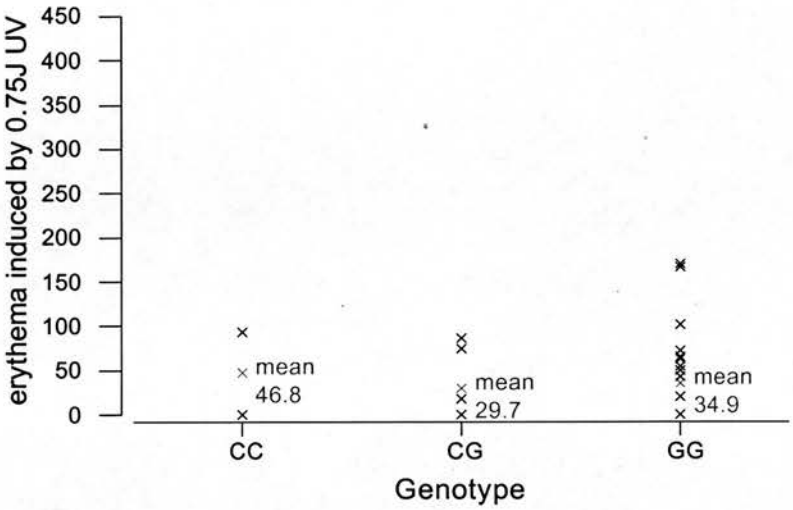


Figure 91. Erythema induced by 0.75J per cm^2 UV by XPG exon 15 genotype.
UV on inner forearm, measured at 24 hours

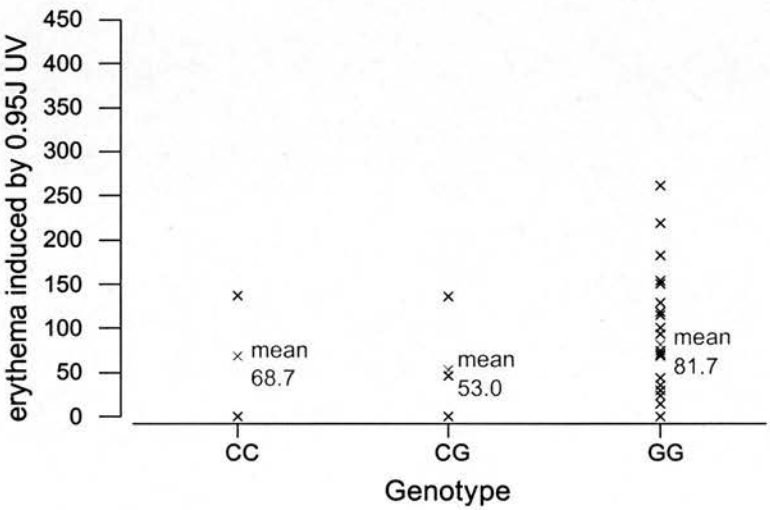


Figure 92 Erythema induced by 0.95J per cm^2 UV by XPG exon 15 genotype.
UV on inner forearm, measured at 24 hours

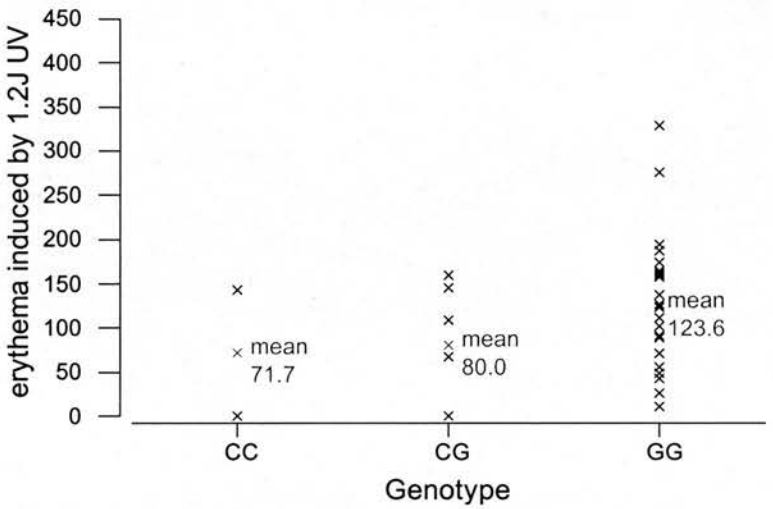


Figure 93. Erythema induced by 1.2J per cm^2 UV by XPG exon 15 genotype.

UV on inner forearm, measured at 24 hours

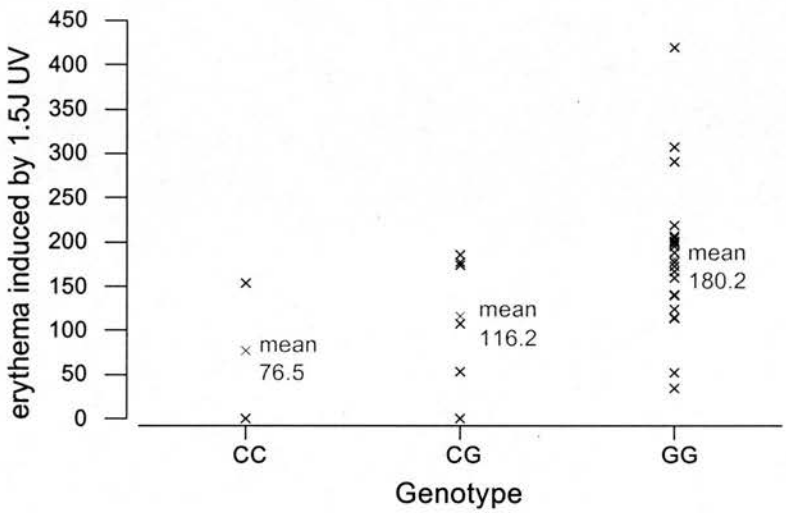


Figure 94 Erythema induced by 1.5J per cm^2 UV by XPG exon 15 genotype.

UV on inner forearm, measured at 24 hours

Analysis of XPG exon 15 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	2.50	3.54	2.50
CG	6	0.333	0.816	0.333
GG	23	5.78	19.16	4.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	150	75	0.26	0.773
Error	28	8096	289		
Total	30	8246			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	0.875	1.808	0.639
GG	23	5.78	19.16	4.00

Source	DF	SS	MS	F	P
Factor	1	143	143	0.51	0.480
Error	29	8103	279		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	26.8	38.0	26.8
CG	6	15.2	26.8	11.0
GG	23	17.43	38.91	8.11

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P

Factor	2	207	103	0.08	0.927
Error	28	38349	1370		
Total	30	38555			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	18.08	27.37	9.68
GG	23	17.43	38.91	8.11

Source	DF	SS	MS	F	P
Factor	1	143	143	0.51	0.480
Error	29	8103	279		
Total	30	8246			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	46.8	66.2	46.8
CG	6	29.7	39.8	16.2
GG	23	34.9	52.1	10.9

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	448	224	0.09	0.917
Error	28	71960	2570		
Total	30	72408			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	34.0	42.7	15.1
GG	23	34.9	52.1	10.9

Source	DF	SS	MS	F	P
Factor	1	5	5	0.00	0.963
Error	29	72402	2497		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	68.7	97.1	68.7
CG	6	53.0	66.7	27.2
GG	23	81.7	75.0	15.6

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	4026	2013	0.36	0.699
Error	28	155324	5547		
Total	30	159349			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	56.9	67.7	23.9
GG0	23	81.7	75.0	15.6

Source	DF	SS	MS	F	P
Factor	1	3657	3657	0.68	0.416
Error	29	155692	5369		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	71.7	101.3	71.7
CG	6	80.0	69.7	28.4
GG	23	123.6	79.6	16.6

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	12512	6256	1.01	0.378
Error	28	174076	6217		
Total	30	186588			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	77.9	70.4	24.9
GG	3	123.6	79.6	16.6

Source	DF	SS	MS	F	P
Factor	1	12408	12408	2.07	0.161
Error	29	174180	6006		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	2	76.5	108.2	76.5
CG	6	116.2	76.8	31.4
GG	23	180.2	81.6	17.0

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	34834	17417	2.60	0.092
Error	28	187608	6700		
Total	30	222442			

Comparison of CC and CG genotypes together with GG homozygotes:

Genotype	N	Mean	StDev	SE Mean
CC/CG	8	106.3	78.9	27.9
GG	23	180.2	81.6	17.0

Source	DF	SS	MS	F	P
Factor	1	32474	32474	4.96	0.034

Error	29	189968	6551
Total	30	222442	

In group 2, the same pattern was observed (except at 0.6J per cm^2) of the GG genotype having higher levels of erythema, which is thus more susceptible to UVR, than the CC and CG genotypes. An increased susceptibility to UVR could be due to the GG genotype being less able to repair DNA damage induced by UV, which would in turn lead to an increases risk of cancer. This was only significant at the highest UV dose examined though, 1.5J per cm^2 ($p=0.034$). Although no significant association was observed at the other UV doses, it is encouraging that it was the highest dose examined that was significant, as this would cause the greatest amount of DNA damage. As was the case in group 1, although the association between XPG exon 15 and erythemal response was significant at 1.5J per cm^2, the genotype does not explain all of the variation in erythema values, with most due to other factors.

In group 2, n=10 was used to determine the power of a balanced test, and n=2 used for the power of the least balanced test. At 0.47J, the between variance was 75, and within variance 289, which gave a power of 0.47 when n=10, and 0.08 when n=2. At 1.5J, the between variance of 17417 and within variance of 6700 gave power of 0.99 when n=10, and 0.39 when n=2. As in group 1m higher power values are observed at higher UV doses, this is also where significant difference between genotypes was observed. The power of these tests would need to be increased in order to fully examine the effect of the polymorphism.

The lower frequency of chromosomal aberrations seen in cells which were CC genotype compared with cells which were CG and GG (although not significant) in the study by Vodicka and colleagues (2004) strengthens the reasoning that the GG genotype could be more susceptible to DNA damage and hence at an increased risk of cancer, as chromosomal aberrations are correlated with onset of cancer. The same group also measured DNA repair capacity of lymphocytes after gamma ray irradiation, as determined by the number of single strand breaks in the DNA measured by a Comet assay. XPG exon 15 CC homozygotes were found to have a moderately higher DNA repair rate than GG homozygotes, which again, although not significant ($p=0.089$) is of

interest, as if the CC homozygotes are more efficient at repairing DNA damage, they are likely to be less susceptible to DNA-damaging agents, such as UVR, which would provide some support for the hypothesis investigated here, that the GG genotype is more susceptible to UVR.

Jeon *et al* (2003) found the GG genotype at a lower frequency in lung cancer patients than in healthy controls, and suggested that the GG genotype might confer a decreased risk of lung cancer than the CC or CG genotypes. As NER removes both UV-induced photoadducts and DNA adducts induced by tobacco carcinogens, it would be expected that if the XPG exon 15 association was associated with susceptibility to cancer, the same genotype would confer protection in different cancer types, which is not seen here. The different genotype frequencies of the exon 15 polymorphism by geographical location might impinge on results seen in fairly small studies on a homogenous population.

As significant associations were seen in both study groups, with the GG genotype being more susceptible to UV, it was decided to analyse further polymorphisms along the XPG gene, to determine if an extension of this association was observed.

Additional XPG SNPs analysed

In order to see if the above association extended along the XPG gene, three additional SNPs were investigated. These were chosen from the NCBI SNP database. Factors taken into consideration when choosing snps were the position of the snp in relation to the exon 15 polymorphism and the reported allele frequency. Many of the snps had an extremely low variant allele frequency. The variant alleles with low reported frequencies would not necessarily have been picked up in a study of this size, therefore these polymorphisms were ignored.

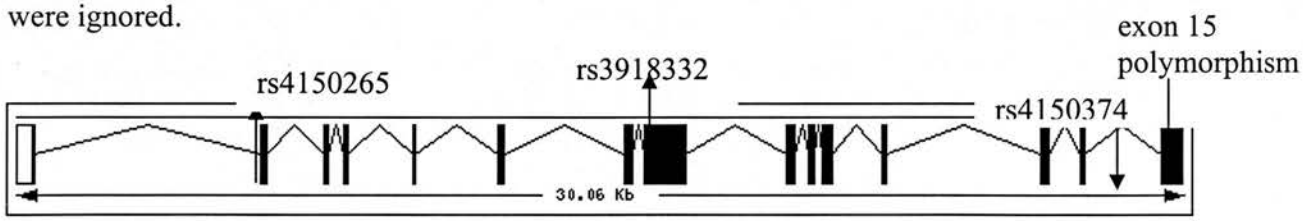


Figure 95 The XPG gene, showing the 15 exons, with positions of additional SNPs analysed.

rs4150265

The SNP rs4150265 (also identified as rs1047768) consists of a C to T polymorphism, and is located in exon 2 of XPG. The polymorphism leads to a nonsynonymous amino acid change. An RFLP assay was used to genotype samples for the rs4150265 polymorphism. A NcoI recognition site exists in the 377bp PCR product in the presence of the T allele, and is lost when there is C. The CC homozygote is undigested, and results in a 377bp fragment. The TT homozygote yields two fragments, of 254 and 123 bp (123bp fragment not seen in gel below). The heterozygous CT therefore results in fragments of 377, 254 and 123 bp.

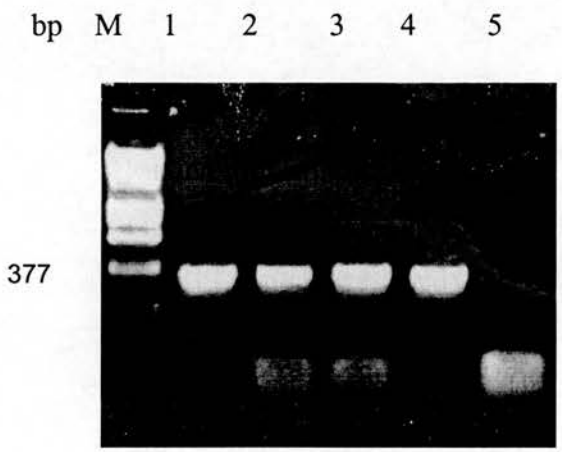


Figure 96 , rs4150265 PCR
Lanes 1-4, PCR product
Lane 5, negative control

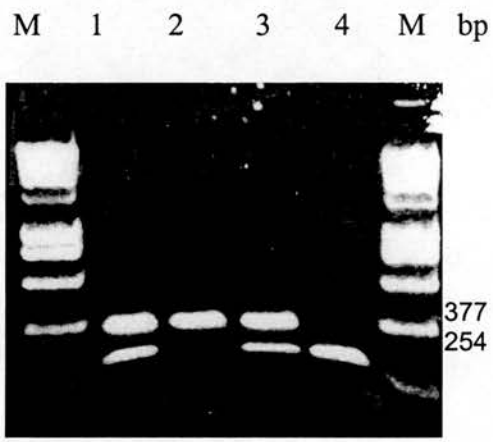


Figure 97 , rs4150265 digest
Lanes 1, 3, CT
Lane 2, CC
Lane 4, TT

rs4150265 Genotype frequencies

Genotype	Group 1	Group 2
CC	30/73 (41.10%)	9/31 (29.03%)
CT	31/73 (42.46%)	12/31 (38.71%)
TT	12/73 (16.44%)	10/31 (32.26%)
Total (100%)	73 (100%)	31 (100%)

No statistically significant difference was observed in genotype frequencies between the two study groups (χ^2 DF = 2, P-Value = 0.174)

Erythema induced by incremental doses of UVR by rs4150265 genotype in group 1.

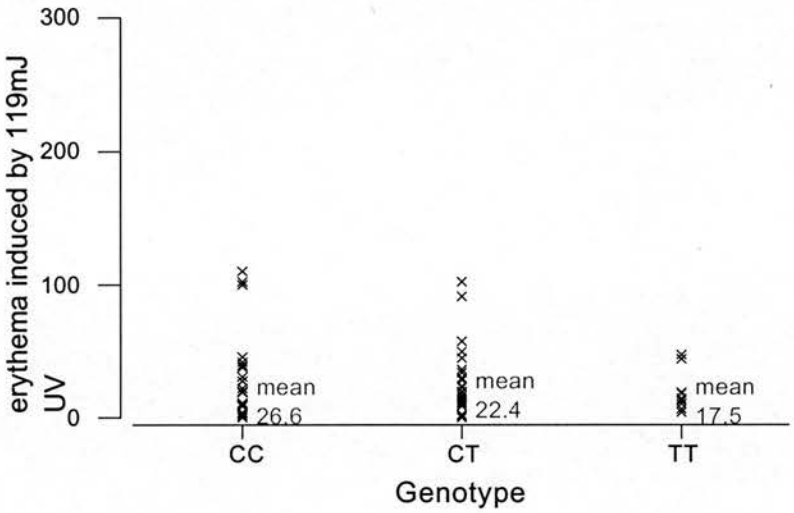


Figure 98. Erythema induced by 119mJ per cm^2 UV by rs4150265 genotype.

UV on lower back, measured at 48 hours.

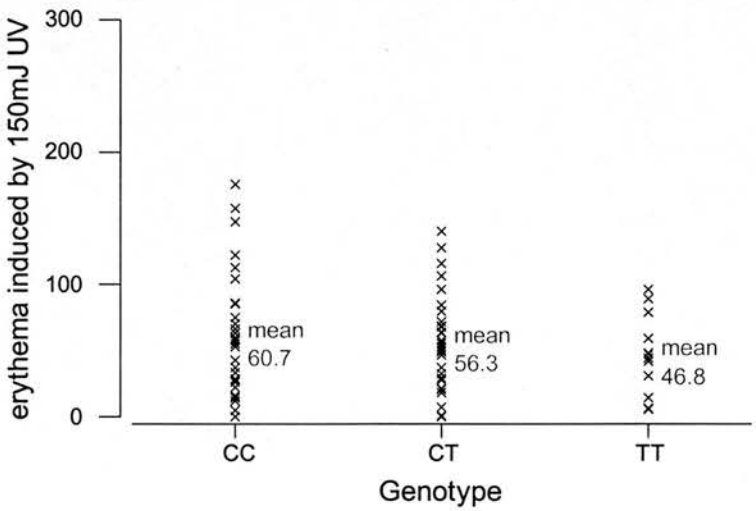


Figure 99 Erythema induced by 150mJ per cm^2 UV by rs4150265 genotype.

UV on lower back, measured at 48 hours

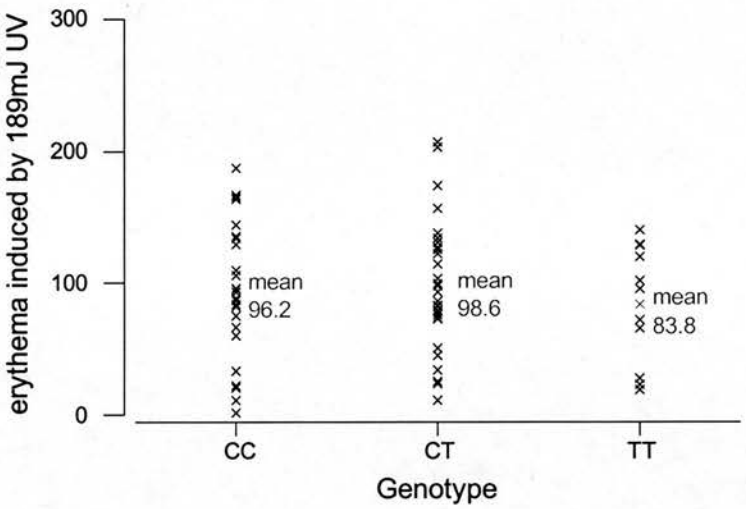


Figure 100 Erythema induced by 189mJ per cm^2 UV by rs4150265 genotype.
UV on lower back, measured at 48 hours.

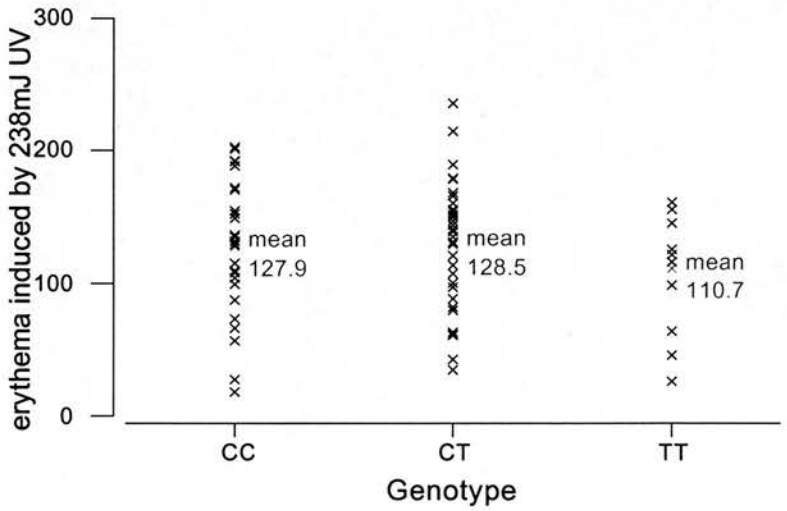


Figure 101. Erythema induced by 238mJ per cm^2 UV by rs4150265 genotype.
UV on lower back, measured at 48 hours.

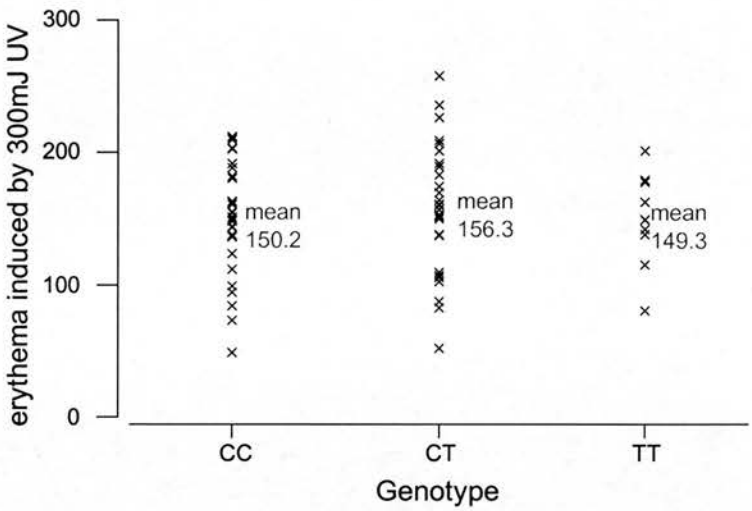


Figure 102. Erythema induced by 300mJ per cm^2 UV by rs4150265 genotype.
UV on lower back, measured at 48 hours.

Analysis of rs4150265 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	30	26.59	29.62	5.41
CT	31	22.51	25.35	4.55
TT	12	17.62	14.52	4.19

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	731	365	0.54	0.583
Error	70	47037	672		
Total	72	47768			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	30	60.74	46.23	8.44
CT	31	55.93	35.39	6.36
TT	12	48.51	30.86	8.91

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1313	657	0.42	0.660
Error	70	110025	1572		
Total	72	111338			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	30	96.16	50.15	9.16
CT	31	98.14	48.56	8.72
TT	12	86.3	44.1	12.7

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1240	620	0.26	0.770
Error	70	165075	2358		
Total	72	166315			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	30	127.91	48.12	8.78
CT	31	127.24	48.70	8.75
TT	12	115.5	47.9	13.8

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1474	737	0.32	0.730
Error	70	163514	2336		
Total	72	164988			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	30	150.21	41.96	7.66
CT	31	155.41	46.90	8.42
TT	12	152.12	32.96	9.51

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	419	209	0.11	0.893
Error	70	129002	1843		
Total	72	129421			

No statistically significant association was observed between any of the genotypes and the level of UV-induced erythema at any UV dose examined. The TT homozygote displayed slightly lower mean values of erythema at each dose, but this was not significant. P-values ranged from 0.583 to 0.893. Almost none of the variation in erythemal response could be attributed to genotype, for example at 119mJ per cm^2 (the

lowest dose examined, also dose where p-value was lowest) the SS(factor) value was 731, which makes up only a small amount of the SS(total) value of 47768, the SS(error) value of 47037 accounting for almost all the observed variation.

The balanced analysis of variance would contain three groups each with 25 individuals, and the least, three groups each with 12 individuals. N=25 and n=12 were therefore used for power calculations. At 119mJ, the between variance was 365 and the within variance 672, which gave a power of 0.99 when n=25 and 0.88 when n=12. At 300mJ, the between variance was 209 and within variance was 1843. The power values were 0.54 when n=25, and 0.27 when n=12. The power levels are not high enough at 300mJ, and if the experiment were to be repeated, it would be desirable to use larger number of samples in order to increase the power and be able to confidently accept the p values.

Erythema induced by incremental doses of UV R by rs4150265 genotype in group 2.

Group 2

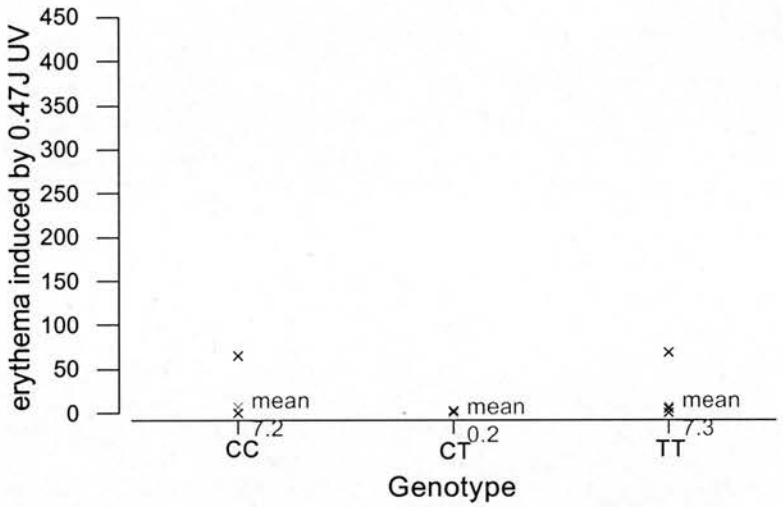


Figure 103 Erythema induced by 0.47J per cm^2 UV by rs4150265 genotype.

UV on inner forearm, measured at 24 hours.

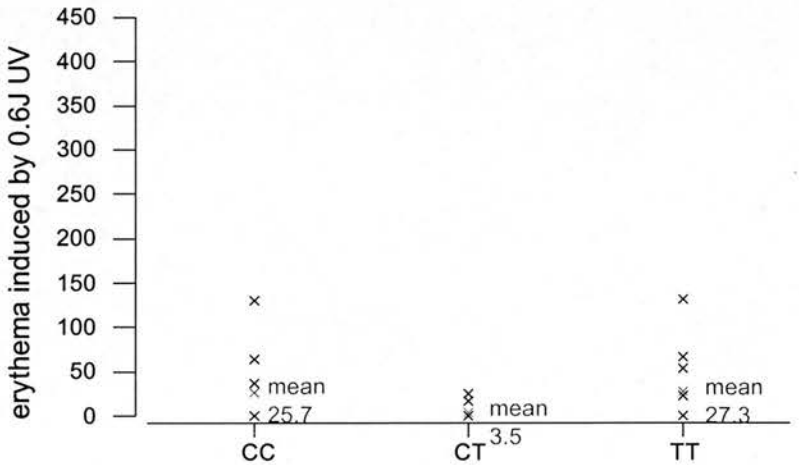


Figure 104 Erythema induced by 0.6J per cm^2 UV by rs4150265 genotype.

UV on inner forearm, measured at 24 hours.

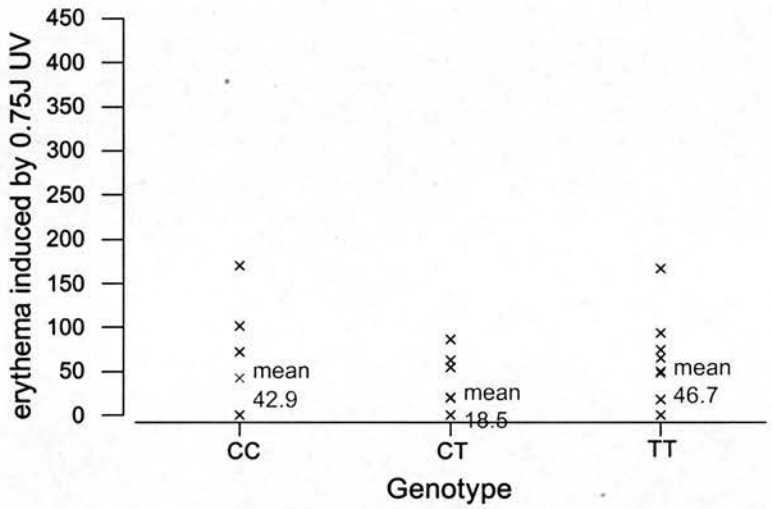


Figure 105 Erythema induced by 0.75J per cm^2 UV by rs4150265 genotype.
UV on inner forearm, measured at 24 hours.

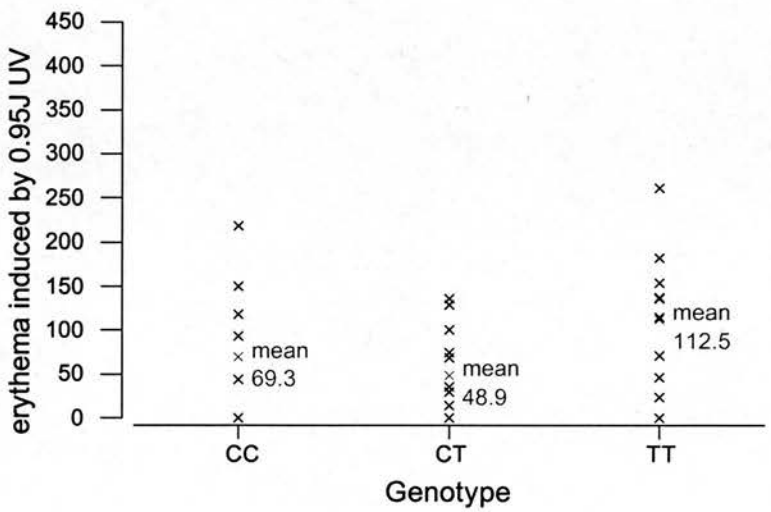


Figure 106 Erythema induced by 0.95J per cm^2 UV by rs4150265 genotype.
UV on inner forearm, measured at 24 hours.

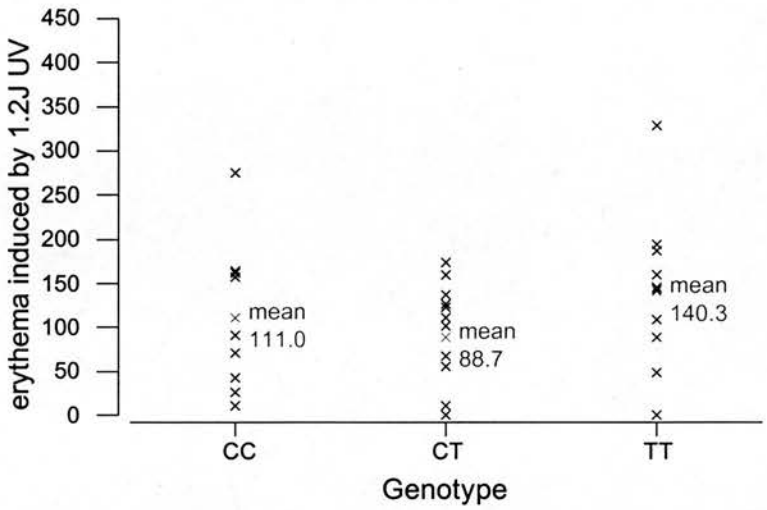


Figure 107 Erythema induced by 1.2J per cm^2 UV by rs4150265 genotype.
UV on inner forearm, measured at 24 hours.

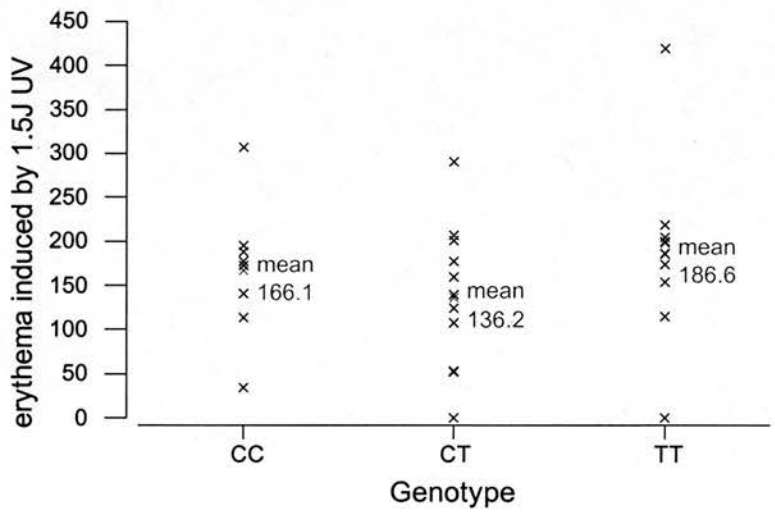


Figure 108. Erythema induced by 1.5J per cm^2 UV by rs4150265 genotype.
UV on inner forearm, measured at 24 hours.

Analysis of rs4150265 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	7.22	21.67	7.22
CT	12	0.167	0.577	0.167
TT	10	7.30	21.39	6.76

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	370	185	0.66	0.525
Error	28	7875	281		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	25.7	45.3	15.1
CT	12	3.50	8.35	2.41
TT	10	27.3	44.0	13.9

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3906	1953	1.58	0.224
Error	28	34650	1237		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	42.9	60.9	20.3
CT	12	18.50	30.82	8.90
TT	10	46.7	54.9	17.3

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	5185	2592	1.08	0.353
Error	28	67223	2401		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	69.3	80.2	26.7
CT	12	48.9	51.3	14.8
TT	10	112.5	79.2	25.0

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	22519	11260	2.30	0.118
Error	28	136830	4887		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	111.0	85.5	28.5
CT	12	88.8	61.2	17.7
TT	10	140.3	89.7	28.4

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14523	7262	1.18	0.322
Error	28	172064	6145		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	9	166.1	72.6	24.2
CT	12	136.2	79.2	22.9
TT	10	186.6	103.8	32.8

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14168	7084	0.95	0.398
Error	28	208274	7438		
Total	30	222442			

In group 2, the rs4150265 genotype was not found to be associated with erythemal response. P values ranged from 0.118 to 0.525. Factor and error values show that very little of the variation is due to genotype.

Power calculations were performed using n=10, and n=9. At 0.47J, the between variance value was 185, and the within variance value 281. These gave a power of 0.88 when n=10, and 0.83 when n=9. At 1.5J, the between variance was 7084 and the within variance 7438, which gave power values of 0.97 when n=10, and 0.94 when n=9. These powers are sufficient to consider the p values which show no significant variation in erythemal response between genotypes to be true.

rs3918332

The SNP rs 3918332 is located within an intron of XPG, between exons 6 and 7. It therefore does not affect the coding sequence. The polymorphism is an A to G change.

No restriction site exists for this polymorphism, so the SNaPshot technique was used to genotype samples.

rs3918332 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
AA	12/69 (17.4%)	9/26 (34.6%)
AG	31/69 (44.9%)	10/26 (38.5%)
GG	26/69 (37.7%)	7/26 (26.9%)
Total (100%)	69 (100%)	26 (100%)

The frequency of the different genotypes of the single nucleotide polymorphism rs3918332 was not statistically significantly different between the two study groups (χ^2, DF = 2, P-Value = 0.188).

Erythema induced by incremental doses of UVR by rs3918332 genotype in group 1.

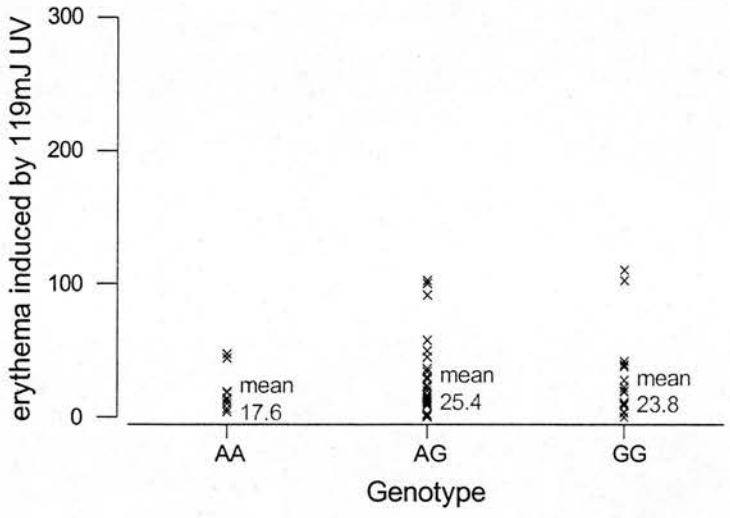


Figure 109 Erythema induced by 119mJ per cm^2 UV by rs3918332 genotype.

UV on lower back, measured at 48 hours

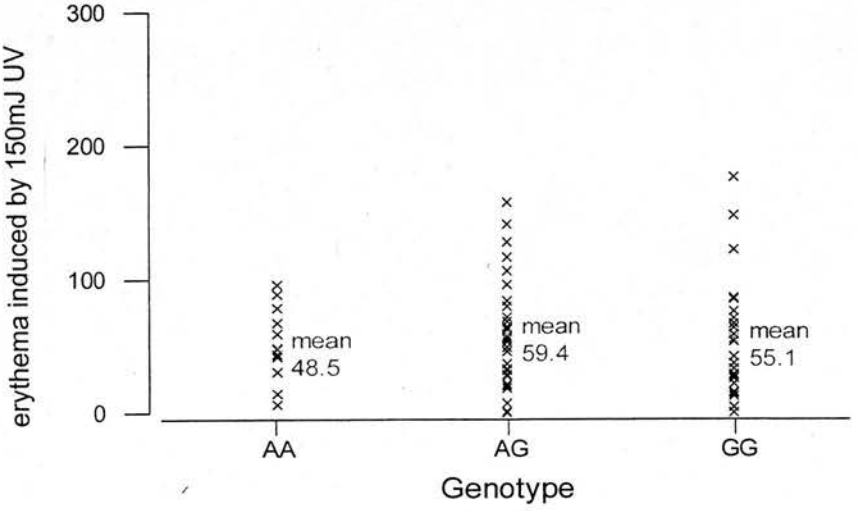


Figure 110 Erythema induced by 150mJ per cm^2 UV by rs3918332 genotype.

UV on lower back, measured at 48 hours

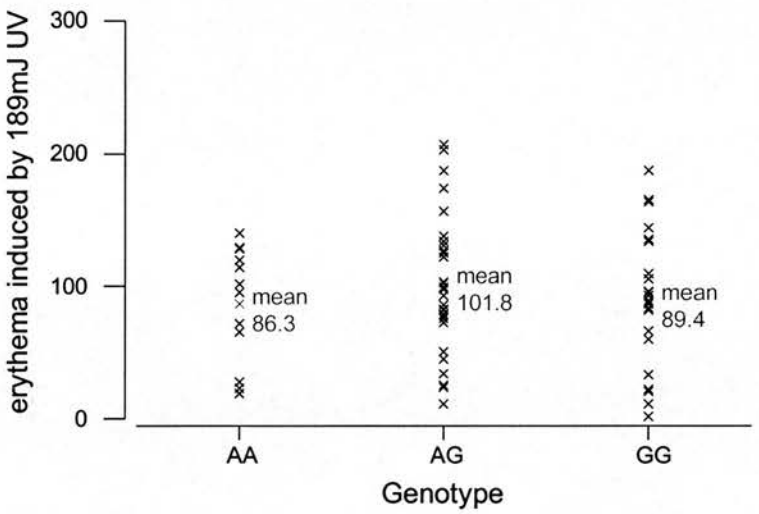


Figure 111. Erythema induced by 189mJ per cm^2 UV by rs3918332 genotype.
UV on lower back, measured at 48 hours

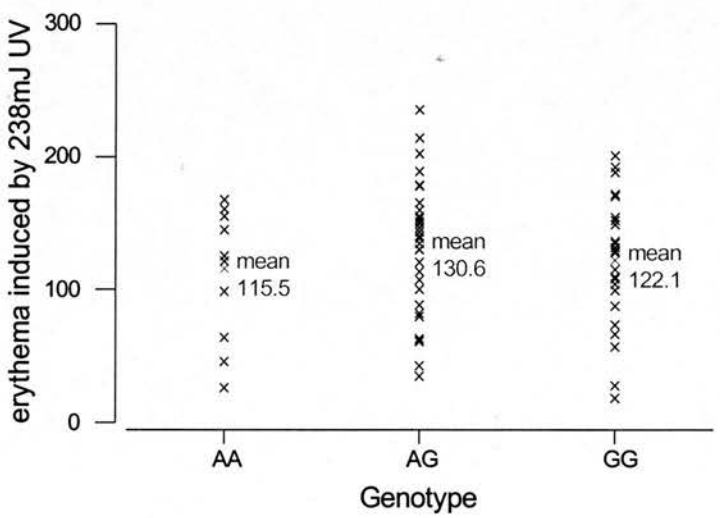


Figure 112 Erythema induced by 238mJ per cm^2 UV by rs3918332 genotype.
UV on lower back, measured at 48 hours

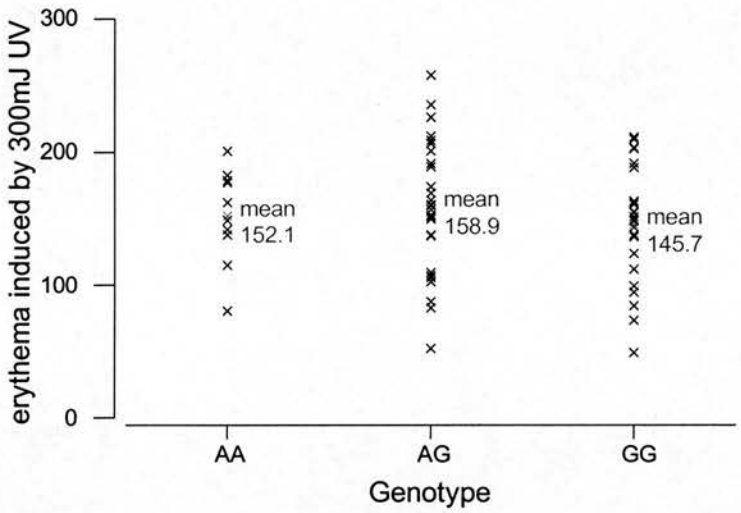


Figure 113 Erythema induced by 300mJ per cm^2 UV by rs3918332 genotype.
UV on lower back, measured at 48 hours

Analysis of rs3918332 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	12	17.62	14.52	4.19
AG	31	25.45	28.79	5.17
GG	26	23.85	27.44	5.38

Analysis of variance of level of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	535	268	0.38	0.683
Error	66	46004	697		
Total	68	46539			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	12	48.51	30.86	8.91
AG	31	59.40	39.78	7.15
GG	26	55.14	42.46	8.33

Analysis of variance of level of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1050	525	0.34	0.716
Error	66	103019	1561		
Total	68	104069			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	12	86.3	44.1	12.7
AG	31	101.83	50.90	9.14

GG	26	89.44	47.67	9.35

Analysis of variance of level of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3134	1567	0.66	0.519
Error	66	155935	2363		
Total	68	159069			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	12	115.5	47.9	13.8
AG	31	130.63	50.16	9.01
GG	26	122.07	47.24	9.26

Analysis of variance of level of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2291	1145	0.48	0.619
Error	66	156509	2371		
Total	68	158800			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	12	152.12	32.96	9.51
AG	31	158.86	47.01	8.44
GG	26	145.69	42.31	8.30

Analysis of variance of level of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2457	1229	0.66	0.521
Error	66	123015	1864		
Total	68	125472			

No association was observed between any of the genotypes and erythemal response to UV at any dose examined. The AA genotype had lower mean values of erythema at all doses except at 300mJ per cm^2, while the heterozygous AG had the highest mean values of erythema. There was no statistical significance between genotypes and erythemal response, with p values ranging from 0.516 to 0.716. In this study group, the SNP rs 3918332 is not associated with erythemal response.

Power calculations were carried out using n=23 (balanced) and n=12 (least balanced). At 119mJ, the between variance value was 268, and the within variance 697. These gave power values of 0.96 when n=23 and 0.74 when n=12. At 300mJ, the between variance value was 1229 and the within variance value 1864, which gave a power of 0.99 when n=23, and 0.93 when n=12. There is sufficient power between these ranges of values to consider that the results obtained with the ANOVA are accurate.

Erythema induced by incremental doses of UVR by rs3918332 genotype in group 2.

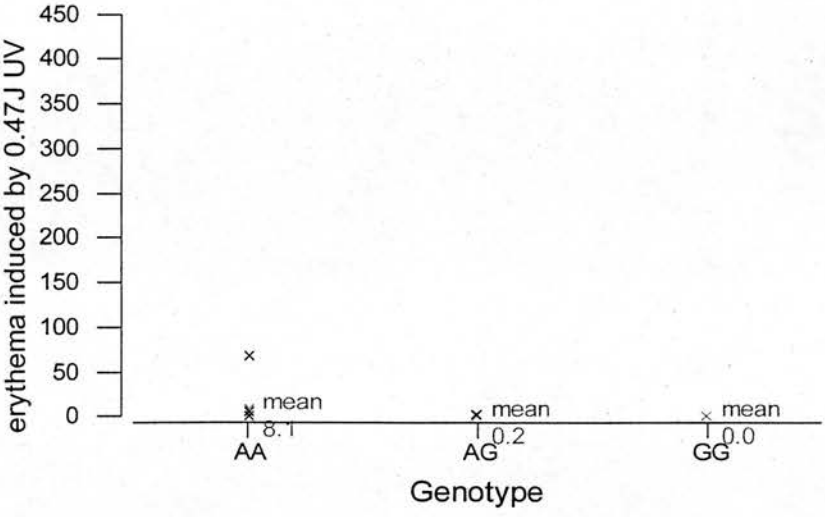


Figure 114 Erythema induced by 0.47J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

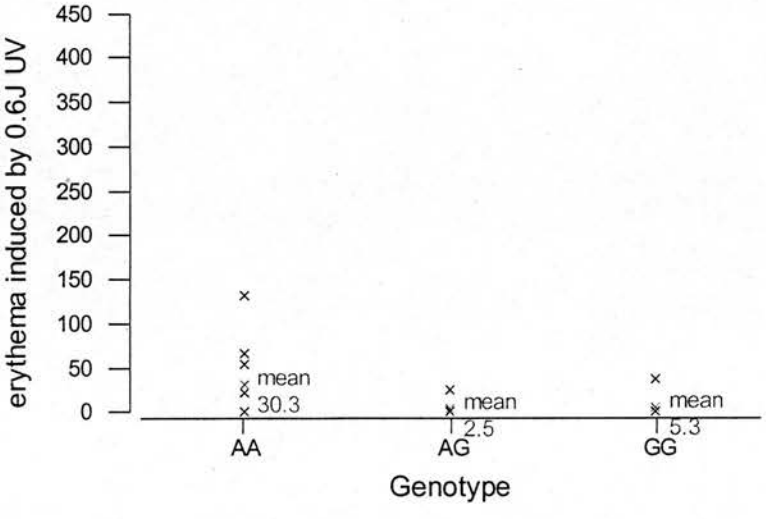


Figure 115 Erythema induced by 0.6J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

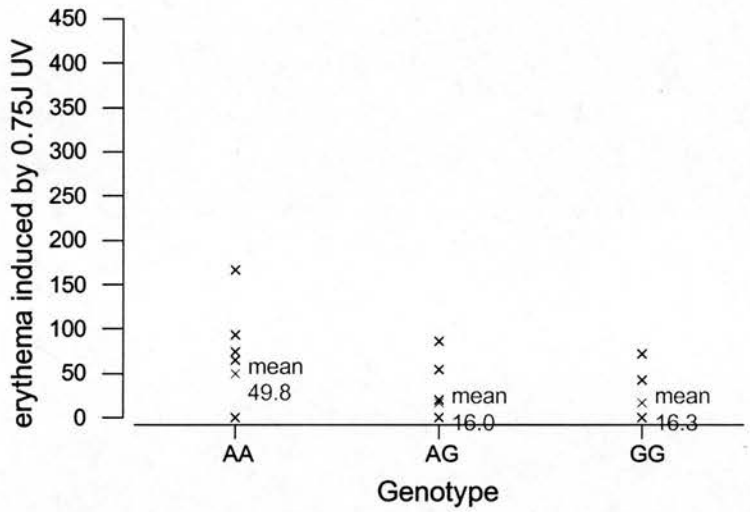


Figure116. Erythema induced by 0.75J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

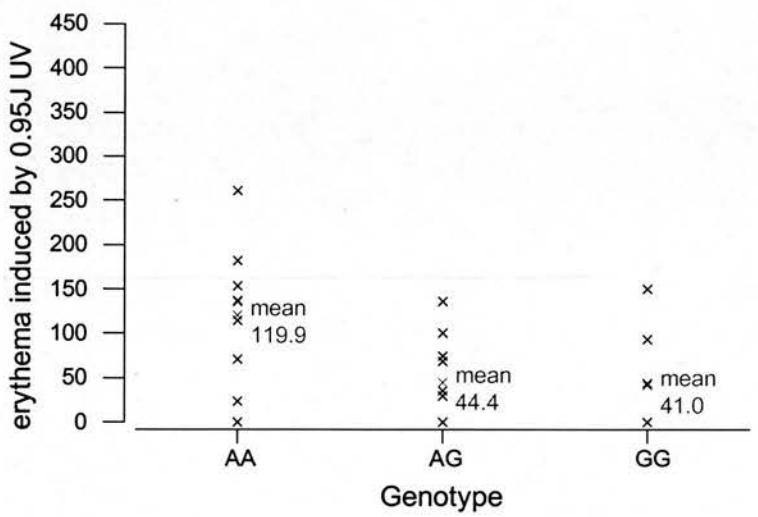


Figure 117 Erythema induced by 0.95J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

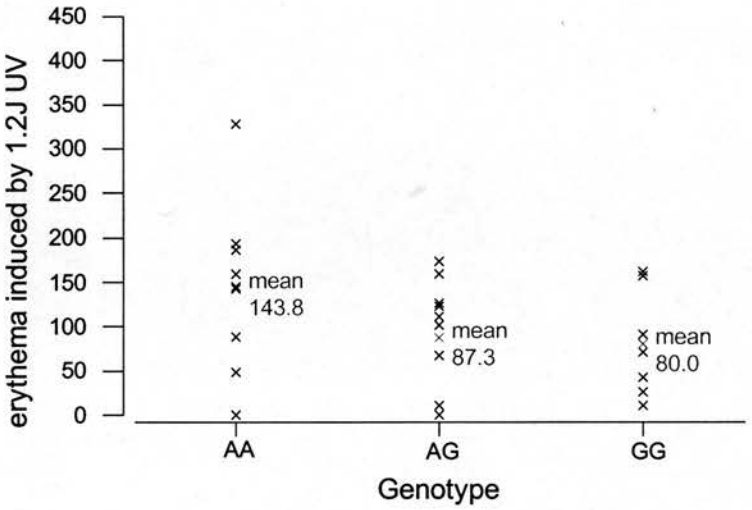


Figure 118 Erythema induced by 1.2J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

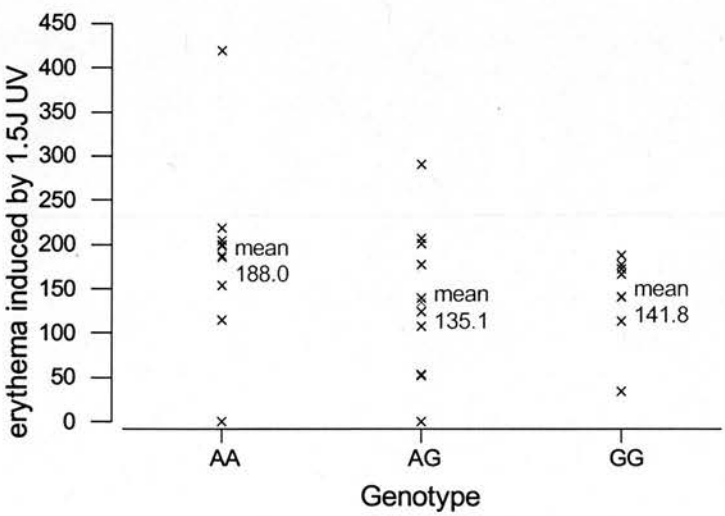


Figure 119 Erythema induced by 1.5J per cm^2 UV by rs3918332 genotype.
UV on inner forearm, measured at 24 hours

Analysis of rs3918332 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	9	8.11	22.52	7.51
AG	10	0.20	0.632	0.200
GG	7	0.00	0.00	0.00

Analysis of variance of level of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	376	188	1.07	0.361
Error	23	4060	177		
Total	2				

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	9	30.3	45.6	15.2
AG	10	2.50	7.91	2.50
GG	7	5.29	13.98	5.29

Analysis of variance of level of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	4211	2106	2.64	0.093
Error	23	18356	798		
Total	25	22568			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean

AA	9	49.9	57.2	19.1
AG	10	16.00	30.10	9.52
GG	7	16.3	29.1	11.0

Analysis of variance of level of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6697	3349	1.95	0.164
Error	23	39407	1713		
Total	25	46104			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	9	119.9	80.3	26.8
AG	10	44.4	48.5	15.3
GG	7	41.0	59.7	22.5

Analysis of variance of level of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	34817	17409	4.26	0.027
Error	23	94009	4087		
Total	25	128826			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	9	143.8	94.4	31.5
AG	10	87.3	64.7	20.5
GG	7	80.0	60.7	22.9

Analysis of variance of level of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	21078	10539	1.85	0.180
Error	23	131110	5700		
Total	25	152188			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	9	188.0	110.0	36.7
AG	10	135.1	87.2	27.6
GG	7	141.8	53.9	20.4

Analysis of variance of level of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14977	7489	0.94	0.404
Error	23	182583	7938		
Total	25	197561			

In group 2, the AA genotype displayed the highest levels of erythema at all doses examined. This was significant at 0.95J per cm^2, but not at any other UV dose. At all doses, including 0.95J per cm^2 where $p<0.05$, and therefore significant, analysis of the factor and error values revealed almost none of the variation in erythemal response can be explained by genotype. If the AA allele were truly associated with an increased sensitivity to UV, it would be expected to reveal itself at all doses, or at least the higher doses where more DNA damage is likely to be caused. This was not the case here. It would that the observed association at 0.95J per cm^2 is due to chance, when factor and error, and p-values at other doses are examined.

Power calculations were carried out, using n=9 (balanced) and n=7. 0.47J gave a between variance value of 188 and within variance value of 177. Power values were 0.96 when n=9 and 0.89 when n=7. 1.5J gave a between variance value of 7489 and a within variance value of 7938. Power values were 0.94 when n=9, and 0.86 when n=7. The power values in this group for this polymorphism were therefore sufficient.

rs4150374

The SNP rs4150374 was the closest snp to the exon 15 polymorphism which was analysed. It has a position in the intron, between exon 14 and 15, and is therefore not a coding change. The polymorphism is an A to G change. No restriction site exists for this polymorphism, therefore the SNaPshot technique was used to genotype samples.

rs4150374 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
AA	45/63 (71.4%)	13/26 (50%)
AG	15/63 (23.8%)	11/26 (42.3%)
GG	3/63 (4.8%)	2/26 (7.7%)
Total (100%)	63 (100%)	26 (100%)

No significant difference in genotype frequencies was observed between the two study groups (χ^2 DF = 2, P-Value = 0.155)

Erythema induced by incremental doses of UVR by rs4150374 genotype in group 1.

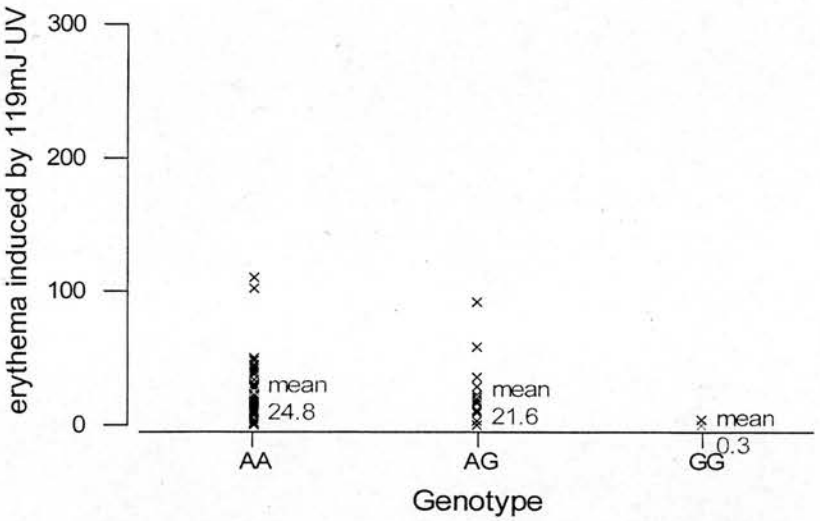


Figure 120. Erythema induced by 119mJ per cm^2 UV by rs4150374 genotype.
UV on lower back, measured at 48 hours.

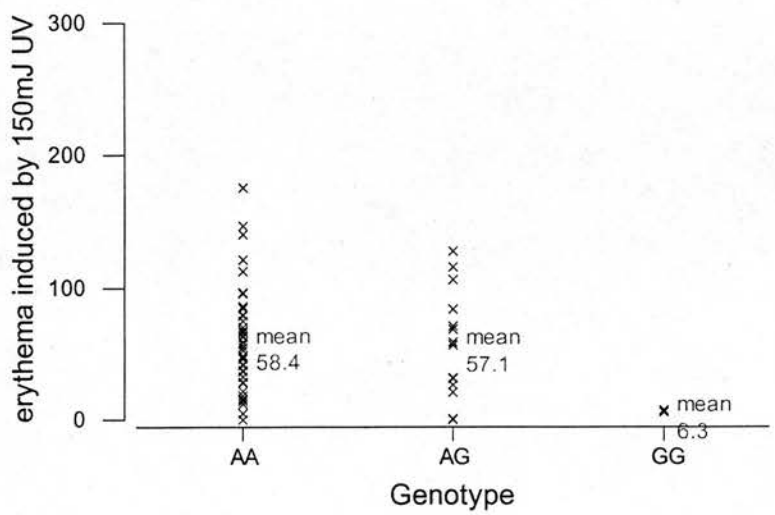


Figure 121 Erythema induced by 150mJ per cm^2 UV by rs4150374 genotype.
UV on lower back, measured at 48 hours.

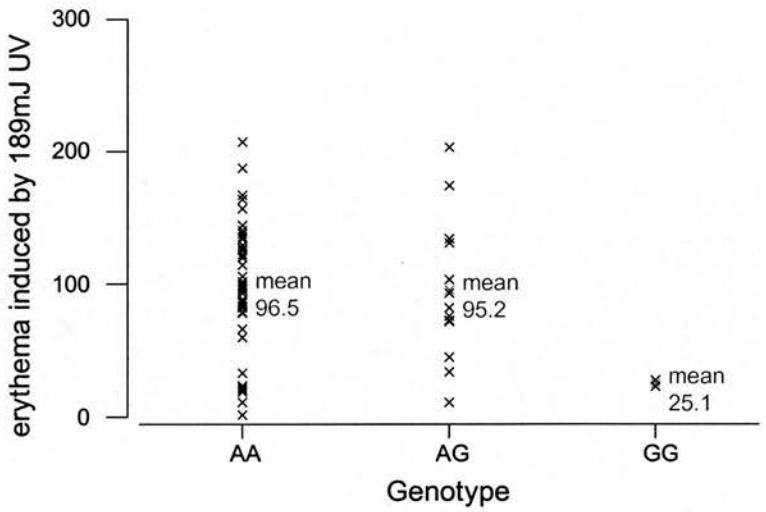


Figure 122 Erythema induced by 189mJ per cm^2 UV by rs4150374 genotype.
UV on lower back, measured at 48 hours.

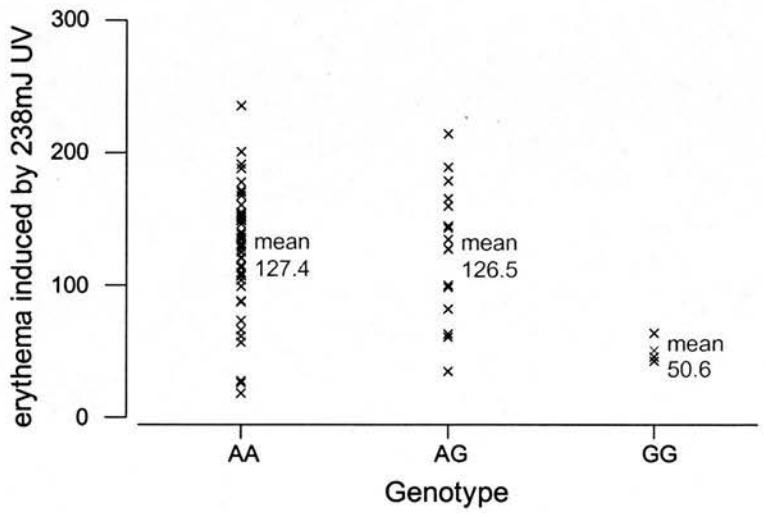


Figure 123 Erythema induced by 238mJ per cm^2 UV by rs4150374 genotype.

UV on lower back, measured at 48 hours.

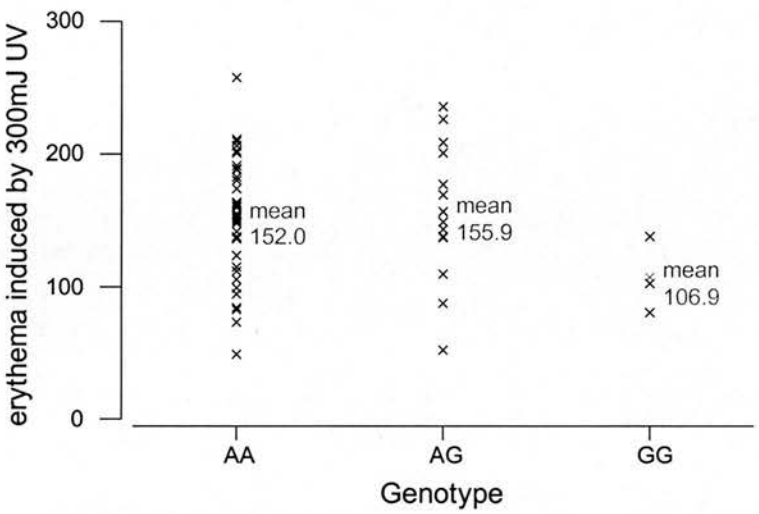


Figure 124 erythema induced by 300mJ per cm^2 UV by rs4150374 genotype

UV on lower back, measured at 48 hours.

Analysis of rs4150374 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	45	24.78	25.89	3.86
AG	15	21.62	24.99	6.45
GG	3	0.33	3.61	2.08

Analysis of variance of level of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1711	855	1.34	0.269
Error	60	38265	638		
Total	62	39976			

AA genotype against combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	45	24.78	25.89	3.86
AG/GG	18	18.07	24.13	5.69

source	DF	SS	MS	F	P
Factor	1	578	578	0.90	0.348
Error	61	39398	646		
Total	62	3997			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	45	58.42	38.24	5.70
AG	15	57.1	39.6	10.2
GG	3	6.33	0.667	0.385

Analysis of variance of level of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7675	3838	2.67	0.077
Error	60	86242	1437		
Total	62	93917			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	45	58.42	38.24	5.70
AG/GG	18	48.66	40.84	9.63

Source	DF	SS	MS	F	P
Factor	1	1224	1224	0.81	0.373
Error	61	92693	1520		
Total	62	93917			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	45	96.49	46.36	6.91
AG	15	95.2	50.5	13.0
GG	3	25.11	2.34	1.35

Analysis of variance of level of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14443	7221	3.33	0.043
Error	60	130286	2171		
Total	62	144729			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	45	96.49	46.36	6.91
AG/GG	18	83.5	53.1	12.5

Source	DF	SS	MS	F	P
Factor	1	2175	2175	0.93	0.338
Error	61	142554	2337		
Total	62	144729			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	45	127.44	46.24	6.89

AG	15	126.5	52.1	13.4
GG	3	50.56	11.59	6.69

Analysis of variance of level of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	16792	8396	3.81	0.028
Error	60	132269	2204		
Total	62	149060			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	45	127.44	46.24	6.89
AG/GG	18	113.8	55.6	13.1

Source	DF	SS	MS	F	P
Factor	1	2387	2387	0.99	0.323
Error	61	146674	2404		
Total	62	149060			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	45	152.03	40.54	6.04
AG	15	155.9	50.1	12.9
GG	3	106.9	29.3	16.9

Analysis of variance of level of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6247	3123	1.72	0.189
Error	60	109234	1821		
Total	62	115480			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	45	152.03	40.54	6.04

AG/GG	18	147.8	50.3	11.8

Source	DF	SS	MS	F	P
Factor	1	236	236	0.12	0.725
Error	61	115245	1889		
Total	62	115480			

When the three genotypes were compared individually, the GG genotype displayed lower mean erythemal responses at all UV doses examined in group 1. This was significant at 189 and 238mJ per cm^2, with p-values of 0.043 and 0.028. The level of significance was approached at 150mJ per cm^2, $p = 0.077$ although this was not formally significant. No association was observed at 119 and 300mJ per cm^2. Analysis of factor and error revealed that most variation could not be explained by genotype. The low number of individuals in the GG genotype group could affect the level of significance; therefore it was decided to combine the AG and GG genotypes.

For group 1, power calculations were carried out using n=21, and n=3. At 119mJ, the between variance was 855 and within variance 638. This gave a power of 0.99 when n=21 and 0.49 when n=3. At 300mJ, the between variance was 3123 and within variance 1821. Power was 1 when n=21, and 0.59 when n=3. The true power will lie between the two values, and it would be preferable to be able to increase the power to be able to be more confident on the association or non-association of this polymorphism and erythemal response.

Erythema induced by incremental doses of UVR by rs4150374 genotype in group 2.

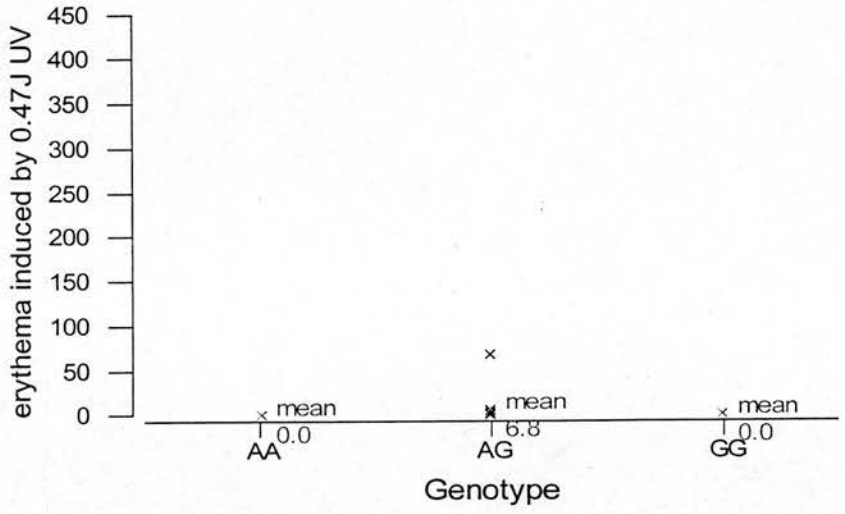


Figure 125. Erythema induced by 0.47J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours.

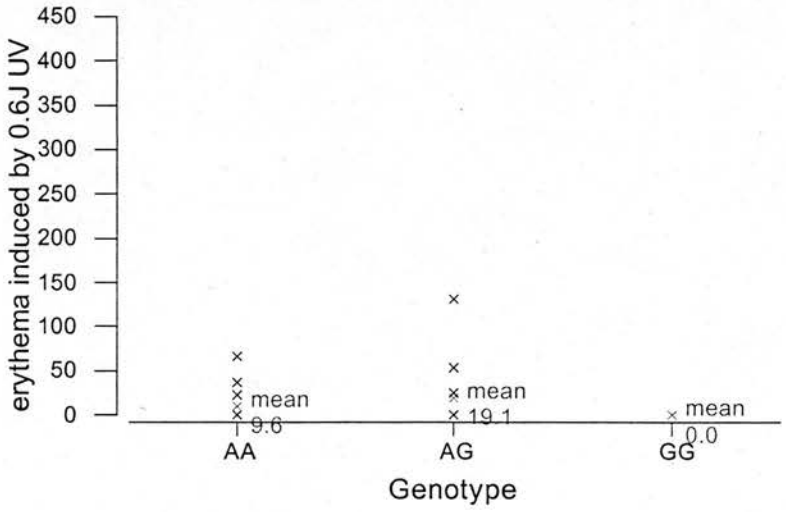


Figure 126. Erythema induced by 0.6J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours.

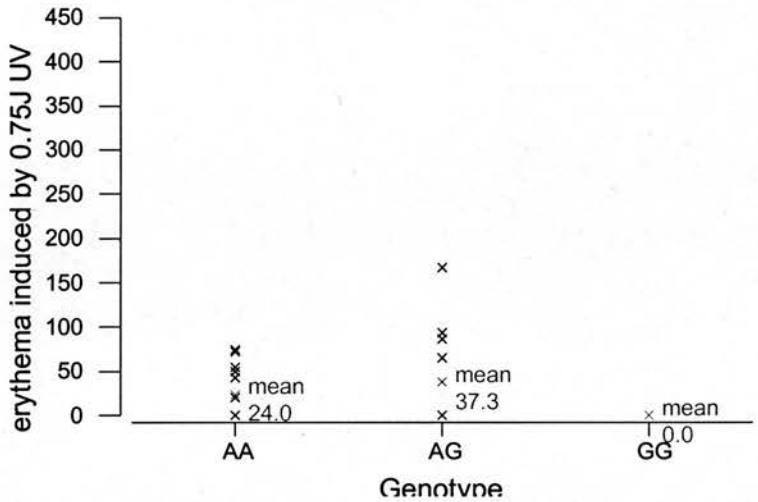


Figure 127 Erythema induced by 0.75J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours.

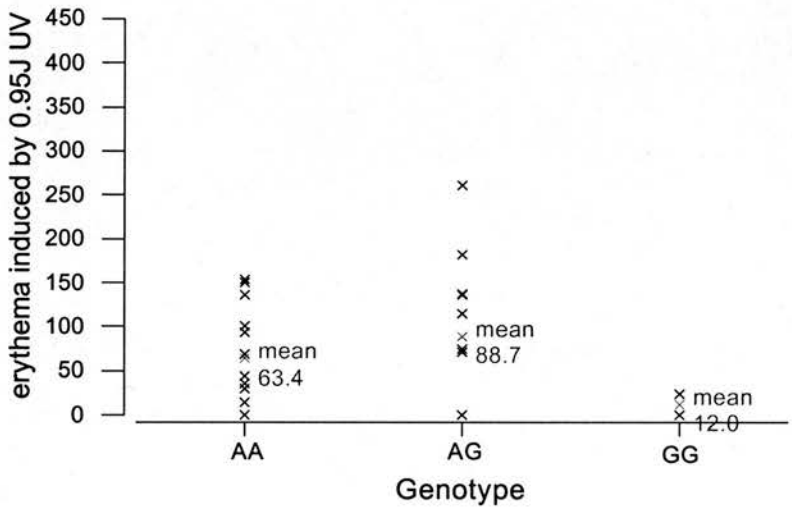


Figure 128 Erythema induced by 0.95J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours.

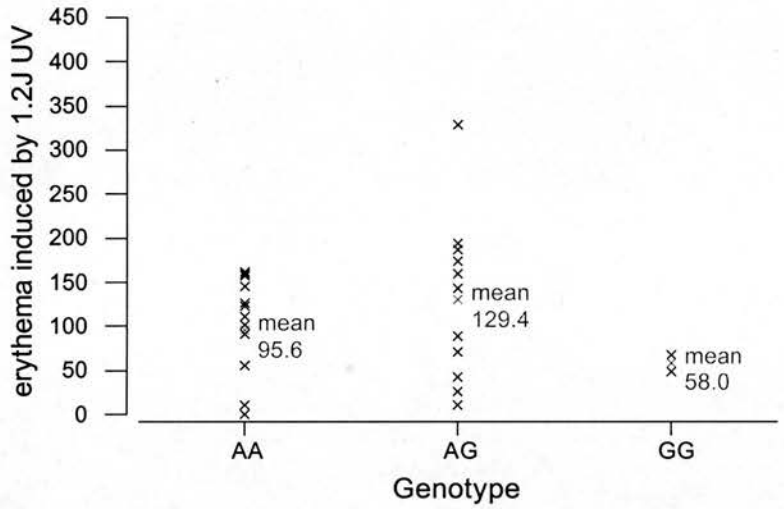


Figure 129 Erythema induced by 1.2J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours

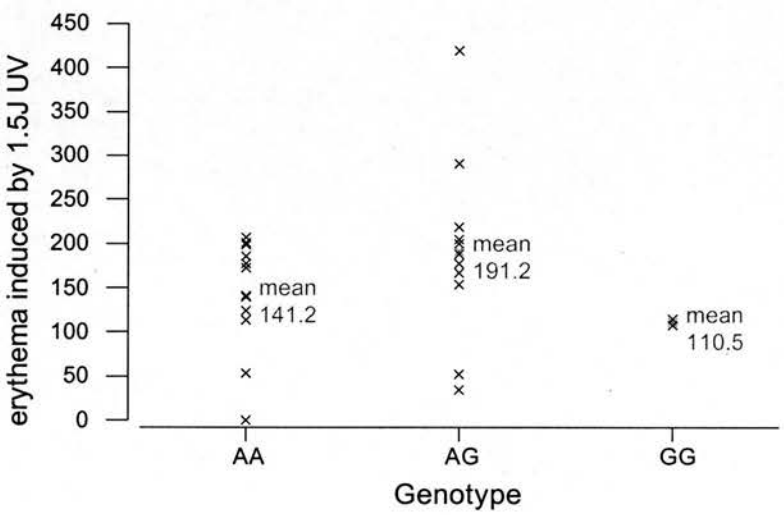


Figure 130. Erythema induced by 1.5J per cm^2 UV by rs4150374 genotype.
UV on inner forearm, measured at 24 hours.

Analysis of rs4150374 genotype and erythemal response to UVR at each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	0.00	0.00	0.00
AG	11	6.82	20.35	6.14
GG	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	295	148	0.82	0.453
Error	23	4142	180		
Total	25	4437			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	0.00	0.00	0.00
AG/GG	13	5.77	18.75	5.20

Source	DF	SS	MS	F	P
Factor	1	216	216	1.23	0.278
Error	24	4220	176		
Total	25	4437			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	9.62	20.43	5.67
AG	11	19.1	40.8	12.3
GG	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	891	445	0.47	0.629
Error	23	21677	942		
Total	25	22568			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	9.62	0.43	5.67
AG/GG	13	16.1	38.0	10.5

source	DF	SS	MS	F	P
Factor	1	276	276	0.30	0.591
Error	24	22292	929		
Total	25	22568			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	24.00	29.93	8.30
AG	11	37.3	57.1	17.2
GG	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2733	1367	0.72	0.495
Error	23	43371	1886		
Total	25	46104			

AA genotype compared with combined AG and GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	24.00	29.93	8.30
AG/GG	13	31.6	54.0	15.0

source	DF	SS	MS	F	P
Factor	1	374	374	0.20	0.662
Error	24	45730	1905		
Total	25	46104			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	63.4	57.7	16.0
AG	11	88.7	87.0	26.2
GG	2	12.0	17.0	12.0

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	11133	5566	1.10	0.348
Error	23	115932	5041		
Total	25	127065			

AA genotype compared with combined AG and GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	63.4	57.7	16.0
AG/GG	13	76.9	84.6	23.5

source	DF	SS	MS	F	P
Factor	1	1178	1178	0.22	0.640
Error	24	125887	5245		
Total	25	12706			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	95.6	60.5	16.8
AG	11	129.4	93.5	28.2
GG	2	58.00	12.73	9.00

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	12003	6002	1.05	0.366
Error	23	131522	5718		
Total	25	143525			

AA genotype compared with combined AG and GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	95.6	60.5	16.8
AG/GG	13	118.4	89.6	24.8

source	DF	SS	MS	F	P
Factor	1	3377	3377	0.58	0.454
Error	24	140148	5839		
Total	25	143525			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
AA	13	141.2	60.9	16.9
AG	11	191.2	104.5	31.5
GG	2	110.50	4.95	3.50

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	20221	10110	1.51	0.241
Error	23	153630	6680		
Total	25	173851			

AA genotype compared with combined AG/GG genotypes:

Genotype	N	Mean	StDev	SE Mean
AA	13	141.2	60.9	16.9
AG/GG	13	178.8	100.1	27.8

source	DF	SS	MS	F	P
Factor	1	9196	9196	1.34	0.258
Error	24	164655	6861		
Total	25	173851			

In group 2, when all three genotypes were compared individually, there was no significant difference in erythemal response at any of the UV doses examined. The GG genotype had the lowest mean levels of erythema at all doses examined, although this was not statistically significant. P-values ranged from 0.629 to 0.241. Analysis of factor and error revealed that very little of the variation in erythema response can be explained by genotype. The low number of GG homozygotes (2/26, 7.7%) could interfere with statistical analysis, therefore it was decided to combine the GG and AG genotype groups, as in group 1, to ask if the presence of the G allele had any affect on erythemal response.

Power calculations were performed for group 2 using n=9 (balanced) and n=2 (least balanced). At 0.47J, the between variance was 148, and within variance 180. Power was

0.91 when n=9, and 0.16 when n=2. 1.5J gave a between variance value of 10110 and a within variance value of 6680, which gave power of 0.99 when n=9 and 0.25 when n=2.

Discussion of XPG and UV-induced erythema

The association observed between the exon 15 polymorphism and sensitivity to UVR did not extend to the additional SNPs studied. The rs4150374 SNP did tend to approach the level of formal significance (p=0.05) at some doses in group 1, with the GG genotype having lower mean erythemal responses. However, the level of significance was never reached.

It was unfortunate that none of the SNPs identified in the NCBI SNP database, which were closer to the exon 15 polymorphism, had allele frequencies which would have made them feasible for analysis in this size of study. A larger study group would be likely to allow for these rare alleles to be detected. One would expect that if there were a true association between the exon 15 polymorphism and sensitivity to UVR that this would be in linkage disequilibrium with SNPs nearby. Analysis of these SNPs would show the association too in this case.

It would be of interest to conduct an investigation into any functional effect the XPG exon 15 polymorphism might have. One way to do so would be to take skin biopsies of irradiated skin and look for sunburn cells in individuals with different genotypes at this polymorphism. Sunburn cells have undergone apoptosis as a result of DNA damage, and the hypothesis would be that if the exon 15 polymorphism does have any functional significance, cells from individuals with the GG genotype, which had higher mean levels of erythema in this study, would have a greater number of sunburn cells than cells from individuals with CC or CG genotypes.

In a recent study the XPG exon 15 polymorphism was analysed for association with melanoma (Blankenburg *et al* (2005). 294 Caucasian patients with malignant melanoma and 375 healthy controls were genotyped. No significant difference was observed in genotype frequencies between the cases and controls (OR 1.168, 95% CI 0.670-2.

044). Analysis of subgroups of the melanoma population compared with all controls also revealed no association of this polymorphism with increased risk of developing multiple primary melanomas (n=28), negative family history of melanoma (n=227), melanoma in

individuals with low numbers of nevi (n=273), melanoma in individuals over fifty years of age (n=144) and melanomas thicker than 1mm (n=126).

XRCC1 exon 10

The XRCC1 exon 10 polymorphism is located at position 28152 on the GenBank entry L34079. This polymorphism consists of a G to A change, resulting in an amino acid change from Arg to Gln at codon 399. A PCR-RFLP assay was used to genotype samples for the XRCC1 exon 10 polymorphism. A PCR product of 154bp was amplified, which contained an MspI restriction site in the presence of the G allele. The A allele does not contain this site, and results in one fragment of 154bp. The G allele yields two fragments, of 93 and 79 bp.

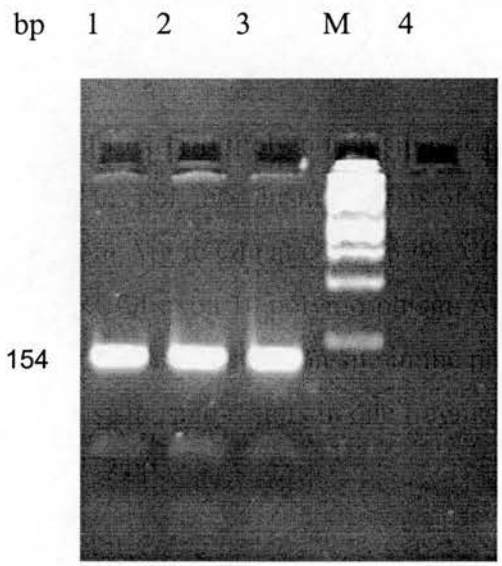


Figure 131, XRCC1 exon 10 PCR products
Lanes 1-3, PCR products
Lane 4, negative control

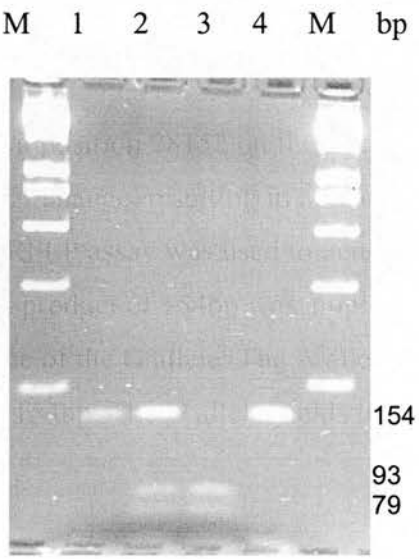


Figure 132, XRCC1 exon 10 digests
Lane 1, AA
Lane 2, GA
Lane 3, GG
Lane 4, undigested PCR product

XRCC1 exon 10 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
GG	19/74 (25.68%)	11/31 (35.48%)
GA	37/74 (50.00%)	17/31 (54.84%)
AA	18/74 (24.32%)	3/31 (9.68%)
Total (100%)	74 (100%)	31 (100%)

The pattern of frequency distribution was similar between the two study groups, with the GA heterozygote genotype being the most frequent in each group, and the variant AA homozygote the least frequent. No significant difference was observed in genotype frequency between the two groups ($\chi 2$ DF = 2, P-Value = 0.204).

Erythema induced by incremental doses of UVR by XRCC1 exon 10 genotype.

UVR on lower back, measured at 48 hours, n=74

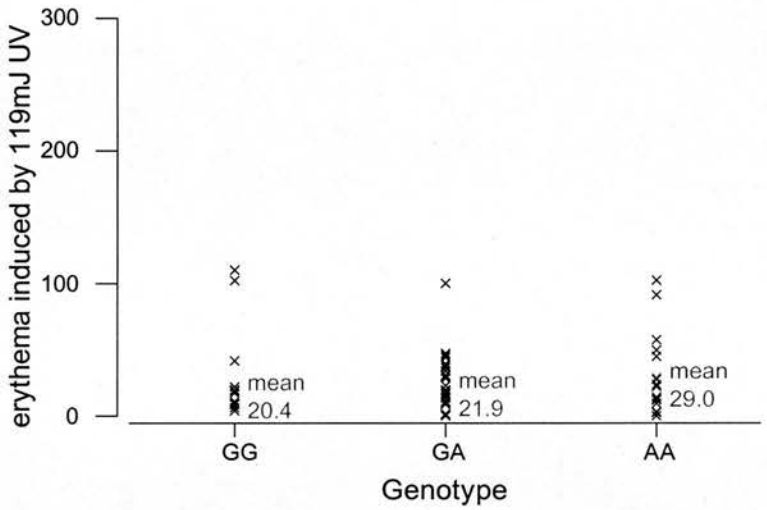


Figure 133 Erythema induced by 119mJ per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 48 hours, n=74.

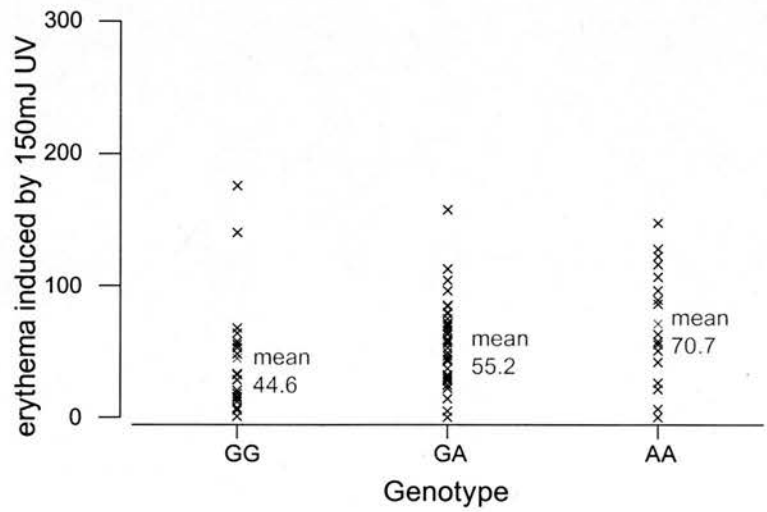


Figure 134 Erythema induced by 150mJ per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 48 hours, n=74.

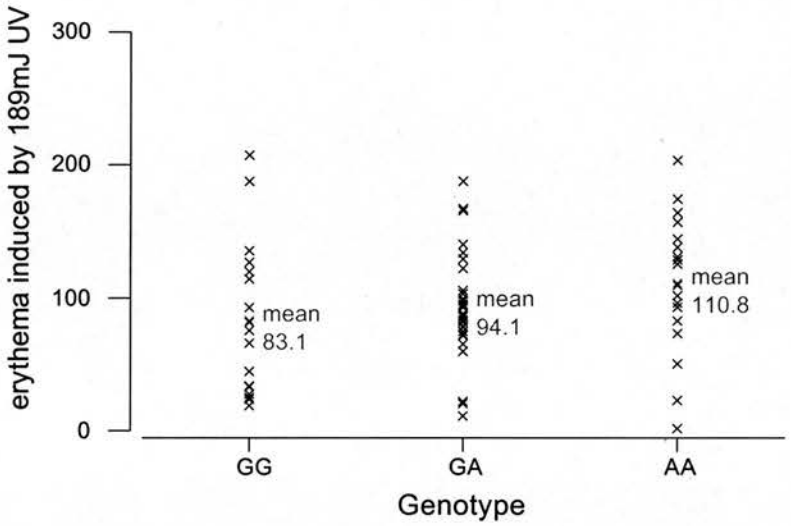


Figure 135. Erythema induced by 189mJ per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 48 hours, n=74.

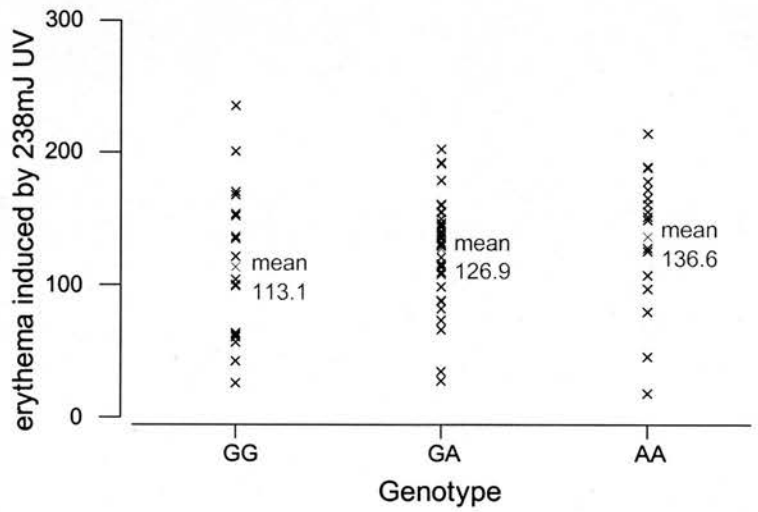


Figure 136 Erythema induced by 238mJ per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 48 hours, n=74.

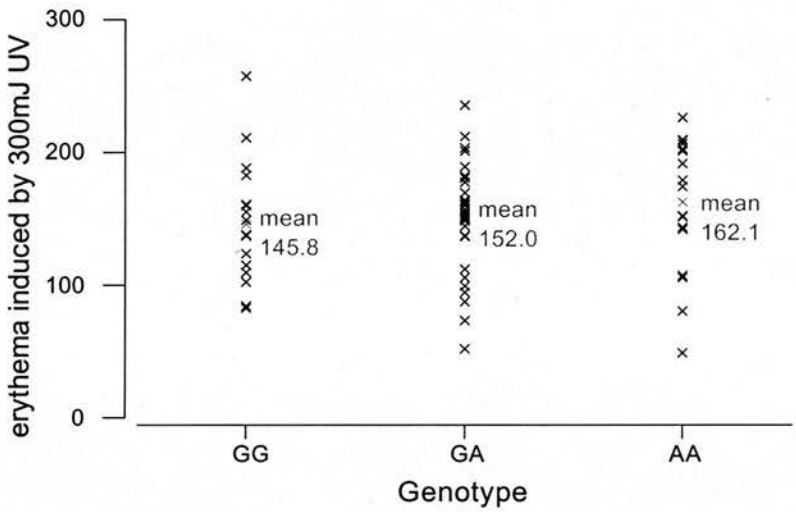


Figure 137. Erythema induced by 300mJ per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of XRCC1 exon 10 genotype and erythemal response to UVR at each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	19	20.40	32.26	7.40
GA	37	21.92	19.05	3.13
AA	18	29.02	29.94	7.06

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	819	409	0.62	0.542
Error	71	47042	663		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	19	44.6	45.4	10.4
GA	37	55.21	32.27	5.31
AA	18	70.7	43.1	10.1

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6338	3169	2.12	0.127
Error	71	106043	1494		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	19	83.2	54.9	12.6
GA	37	94.12	40.36	6.64
AA	18	110.8	52.2	12.3

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7177	3588	1.60	0.209
Error	71	159141	2241		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	19	113.1	57.6	13.2
GA	37	126.91	39.27	6.46
AA	18	136.6	151.3	12.1

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	5194	2597	1.15	0.322
Error	71	159901	2252		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	19	145.78	42.84	9.83
GA	37	151.98	37.81	6.22
AA	18	162.1	50.0	11.8

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2503	1252	0.70	0.500
Error	71	126979	1788		
Total	73	129483			

In group 1, no association was observed between the XRCC1 exon 10 polymorphism and erythemal response at any UV dose examined. P values did not approach significance, and ranged from 0.542 to 0.127. Analysis of factor and error revealed almost all variation between levels of erythema was due to factors other than genotype. The AA genotype displayed higher mean erythemal responses compared with the GG and GA genotypes, but as revealed above, this was no significant.

To analyse the power of these ANOVA tests, power calculations were performed using n=25 (balanced) and n=18 (least balanced). At 119mJ, the between variance was 409 and within variance 663, giving power of 0.99 when n=25 and 0.98 when n=18. 300mJ had a between variance value of 1252 and a within variance value of 1788. Power was 0.99

when n=25 and also when n=18. It can be seen from these values that the power was adequate in this study group.

Erythema induced by incremental doses UVR by XRCC1 exon 10 genotype in group 2.

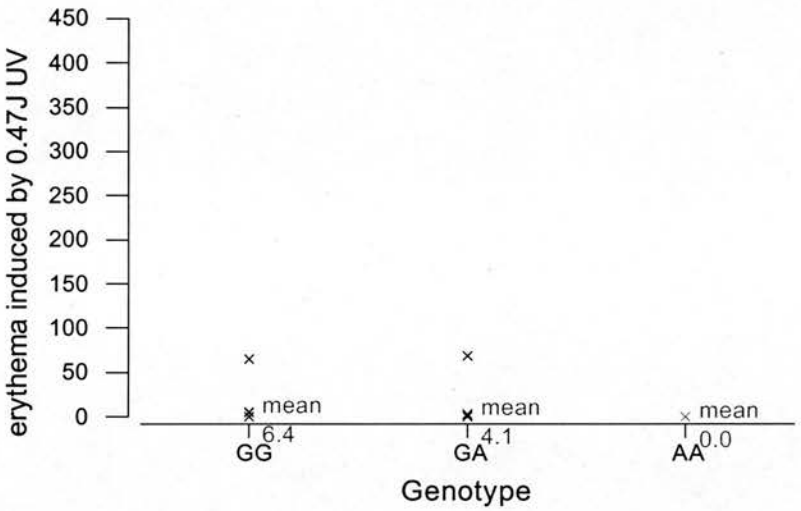


Figure 138 Erythema induced by 0.47J per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

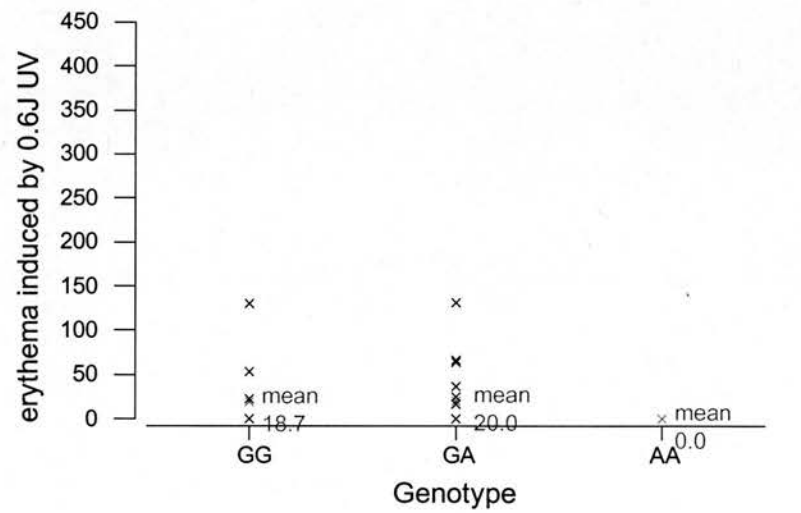


Figure 139. Erythema induced by 0.6J per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

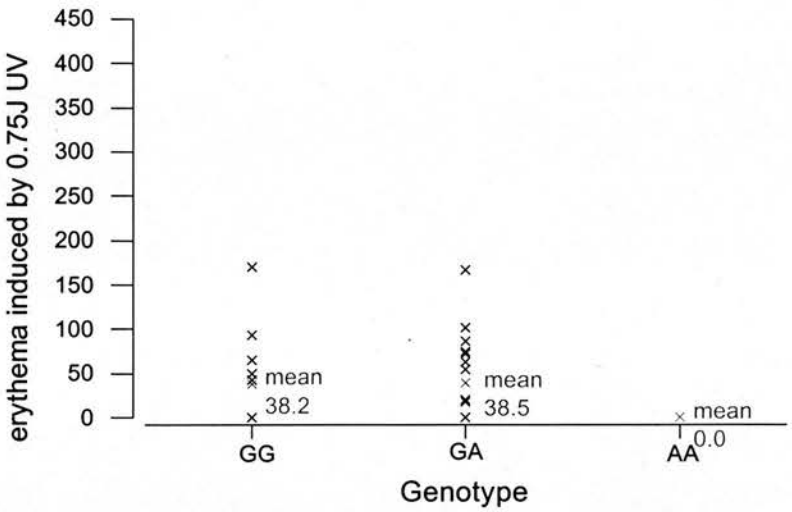


Figure 140 Erythema induced by 0.75 J per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

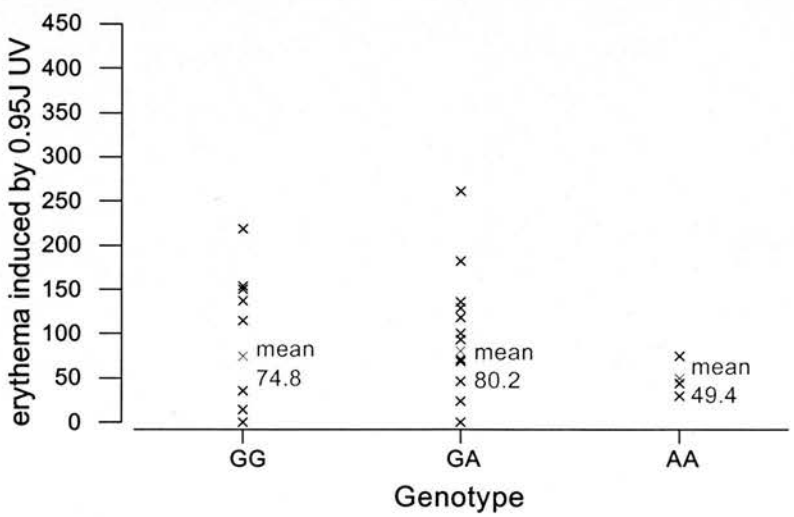


Figure141 Erythema induced by 0.95 J UV per cm^2 by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

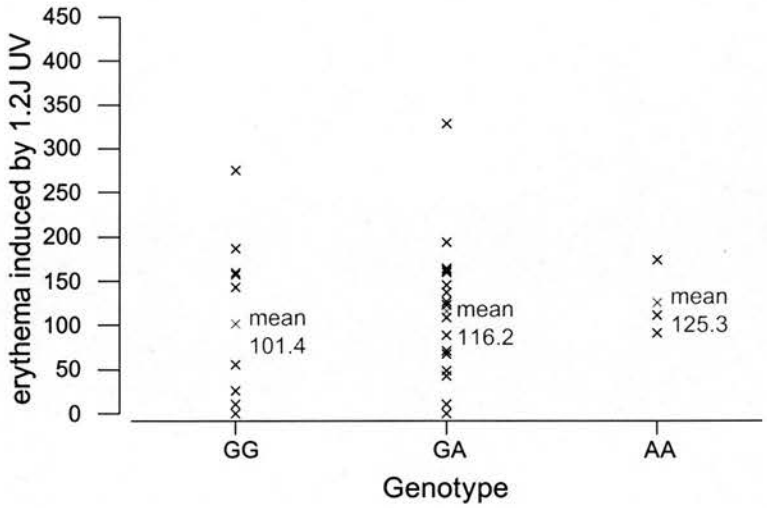


Figure 142 Erythema induced by 1.2 J per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

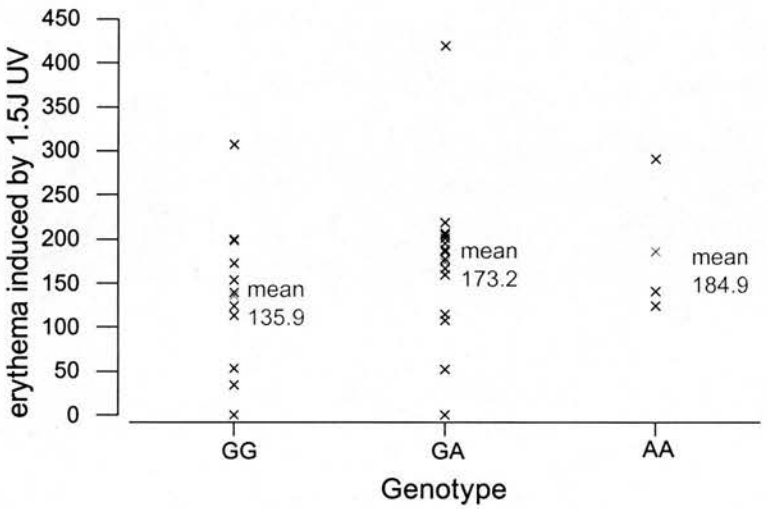


Figure 143 Erythema induced by 1.5 J per cm^2 UV by XRCC1 exon 10 genotype.
UVR on lower back, measured at 24 hours, n=31.

Analysis of XRCC1 exon 10 genotype and erythemal response to UVR at each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	6.36	19.51	5.88
GA	17	4.12	16.47	3.99
AA	3	0.00	0.00	0. 00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	101	51	0.17	0.841
Error	28	8144	291		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	18.7	40.5	12.2
GA	17	20.00	36.31	8.81
AA	3	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1040	520	0.39	0.682
Error	28	37515	1340		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	38.2	54.8	16.5
GA	17	38.5	49.0	11.9
AA	3	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3992	1996	0.82	0.452

Error	28	68416	2443
Total	30	72408	

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	74.8	80.9	24.4
GA	17	80.2	75.2	18.2
AA	3	49.4	23.0	13.3

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2422	1211	0.22	0.807
Error	28	156927	5605		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	101.5	90.7	27.4
GA	17	116.2	78.4	19.0
AA	3	125.3	43.3	25.0

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2052	1026	0.16	0.857
Error	28	184535	6591		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
GG	11	135.9	86.8	26.2
GA	17	173.2	86.2	20.9
AA	3	184.9	92.3	53.3

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P

Factor	2	11165	5582	0.74	0.486
Error	28	211277	7546		
Total	30	222442			

In group 2, no association was observed between the polymorphism and levels of erythema induced at any UV dose examined. The AA genotype displayed higher mean erythema levels than the GG and GA genotypes at the two highest UV doses, but the lowest levels of erythema at all other doses. P values ranged from 0.452 to 0.857, showing no significance.

Power calculations were carried out for group 2 using n=10 (balanced) and n=3 (least balanced). 0.47J gave a between variance value of 51 and a within variance value of 291. These gave a power of 0.33 when n=10, and 0.10 when n=3. 1.5J had a between variance value of 5582 and a within variance value of 7546. These gave a power of 0.91 when n=10 and 0.29 when n=3. The low power shows a need to increase the sample size, which should lead to increase power and more accuracy.

XRCC1 is required for the ligation of newly synthesised DNA to the damaged strand to complete the final stages of the BER pathway. UV induces both direct DNA damage, photoproducts, and indirectly through the production of oxidative stress. The BER pathway repairs oxidised bases. Whether or not an association exists between this polymorphism and cancer risk is the subject of some controversy. The XRCC1 polymorphism has linked to increased risk of various cancers, but also to decreased risk of others. As it is involved with the repair of damage induced by UV, the exon 10 polymorphism was a promising candidate for an association with UV-induced erythemal response.

The AA genotype was reported to lead to a decreased risk of NMSC by Nelson *et al* (2002), OR 0.7; 95% CI 0.4-1.0 for BCC, and OR 0.6; 95% CI 0.3-0.9 for SCC. However, Winsey *et al* (2000) reported no association between the polymorphism and melanoma. As both NMSC and melanoma have UVR in common as a causative agent, these results seem to be in dispute with each other. If the AA genotype did lead to a

decreased risk of NMSC, it might be expected that individuals with this genotype would have lower erythemal responses following exposure to UVR, hence be less sensitive to UV. No significant difference was observed at any UV dose in either study group, implying the exon 10 polymorphism is not involved with the erythemal response to UV, and, by association not involved in the repair of oxidative DNA damage induced by UV. A larger study, investigating the polymorphism in all cutaneous malignancies, and erythemal response could resolve the apparent discrepancies between studies.

XRCC3 exon 7

The XRCC3 exon 7 polymorphism consists of a C to T change at position 18067 on the GenBank entry AF037222, resulting in a Thr to Met amino acid change. When there is a T at this position, a recognition site for NlaIII is present in the PCR product. The C allele does not contain this site, and is not cut by the restriction digest, giving a single fragment of 346bp. The T allele yields two fragments, of 242 and 104bp, with the heterozygous CT yielding all three fragments (104bp fragment not seen on gel below).

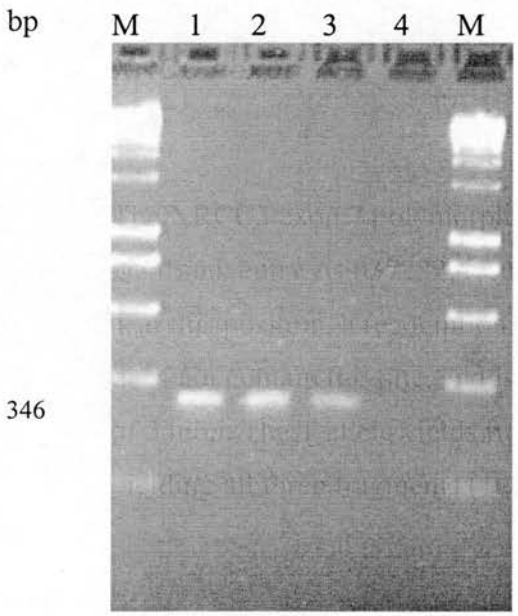


Figure 144 XRCC3 exon 7 PCR digest

Lanes 1-3, PCR products
Lane 4, negative control

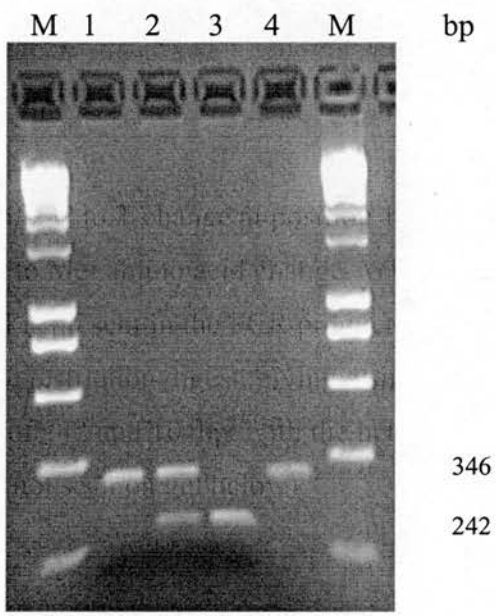


Figure 145, XRCC3 exon 7

Lane 1, CC
Lane 2, CT
Lane 3, TT
Lane 4, undigested PCR product

XRCC3 exon 7 Genotype frequencies

Genotype	Group 1 (%)	Group 2 (%)
CC	27/74 (36.49%)	12/31 (38.71%)
CT	41/74 (55.40%)	17/31 (54.84%)
TT	6/74 (8.11%)	2/31 (6.45%)
Total (100%)	74 (100%)	31 (100%)

No significant difference was observed between genotype frequencies in the two study groups (χ DF = 2, P-Value = 0.947)

Erythema induced by incremental doses of UVR by XRCC3 exon 7 genotype in group 1.

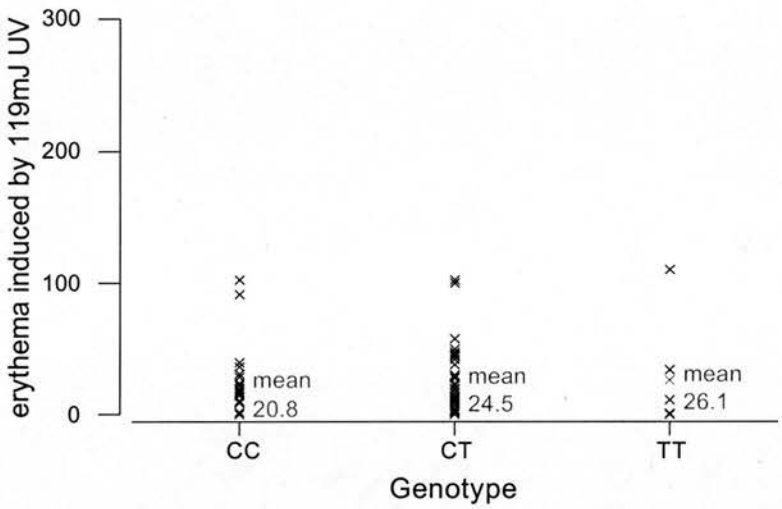


Figure 146 Erythema induced by 119mJ per cm^2 UV by XRCC3 exon 7 genotype.
UVR on lower back, measured at 48 hours, n=74.

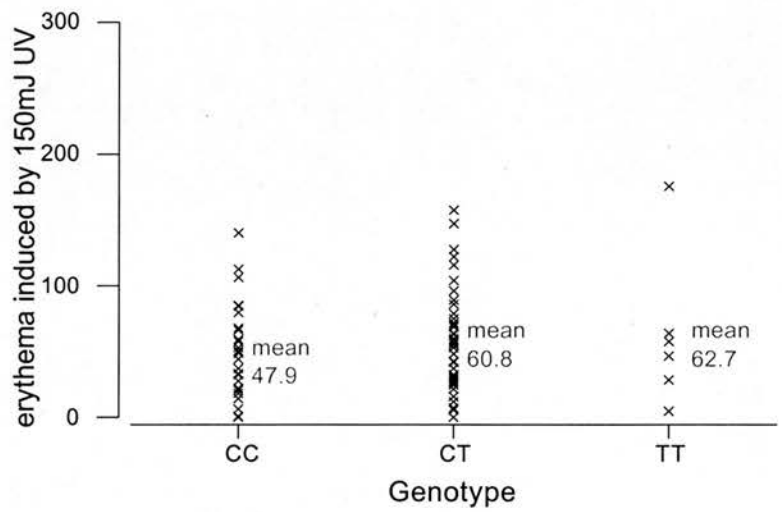


Figure 147 Erythema induced by 150mJ per cm^2 UV by XRCC3 exon 7 genotype.
UVR on lower back, measured at 48 hours, n=74.

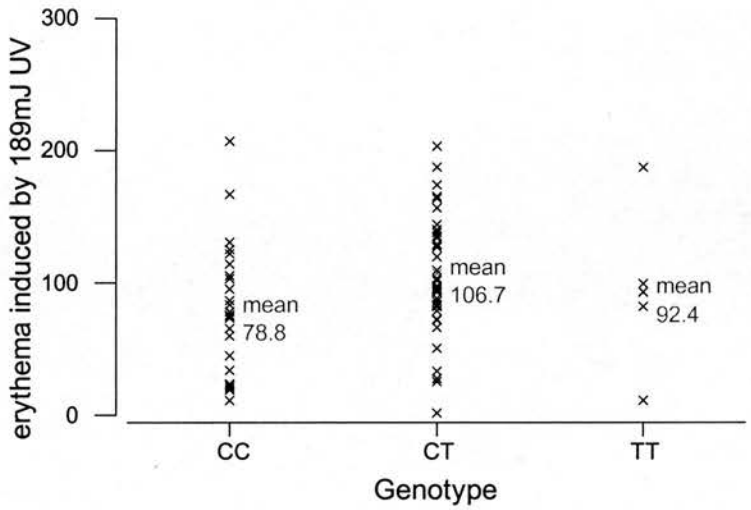


Figure 148 Erythema induced by 189mJ per cm^2 UV by XRCC3 exon 7 genotype.
UVR on lower back, measured at 48 hours, n=74.

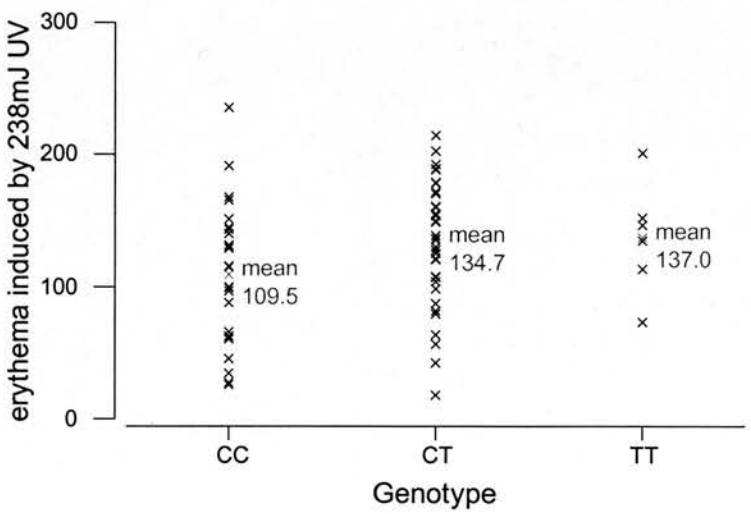


Figure 149. Erythema induced by 238mJ per cm^2 UV by XRCC3 exon 7 genotype.
UVR on lower back, measured at 48 hours, n=74.

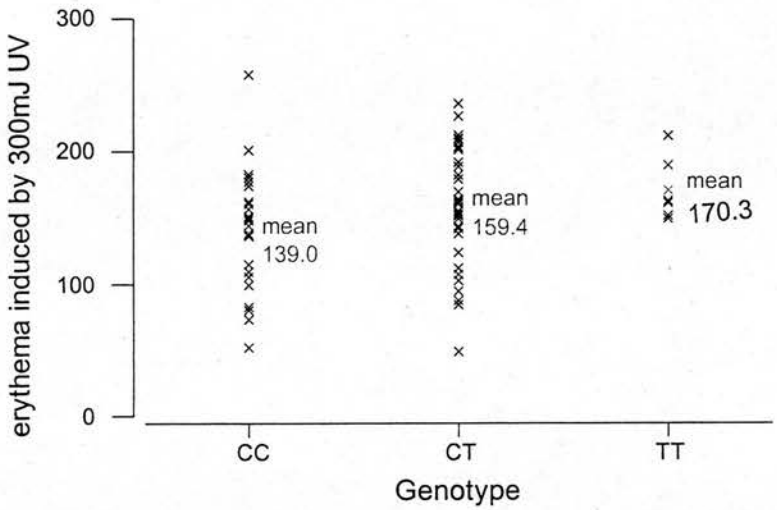


Figure 150. Erythema induced by 300mJ per cm^2 UV by XRCC3 exon 7 genotype.
UVR on lower back, measured at 48 hours, n=74.

Analysis of XRCC3 exon 7 genotype and erythemal response to UVR at each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	27	20.80	24.85	4.78
CT	41	24.47	23.53	3.67
TT	6	26.1	43.4	17.7

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	270	135	0.20	0.818
Error	71	47591	670		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	27	47.92	36.02	6.93
CT	41	60.80	38.12	5.95
TT	6	62.7	59.2	24.2

Analysis of variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2971	1486	0.96	0.386
Error	71	109411	1541		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	27	78.79	47.52	9.15
CT	41	106.70	44.42	6.94
TT	6	92.4	56.5	23.0

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	12742	6371	2.95	0.059
Error	71	153575	2163		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	27	109.50	51.32	9.88
CT	41	134.75	43.70	6.82
TT	6	137.0	42.5	17.3

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	11215	5607	2.59	0.082
Error	71	153880	2167		
Total	73	165094			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	27	138.98	43.69	8.41
CT	41	159.42	41.26	6.44
TT	6	170.28	24.36	9.94

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8784	4392	2.58	0.083
Error	71	120699	1700		
Total	73	129483			

The TT genotype group showed higher mean levels of erythema at all UV doses examined, excluding 189mJ per cm^2, where the heterozygous CT genotype showed the highest mean. This increased level of erythema in TT genotype was not formally significant at any dose ($p=0.05$), but did approach this level of significance at the higher UV doses. The factor and error values reveal that although significance is approached, most of the variation in erythemal response is due to factors other than genotype.

Power calculations were performed using n=25 (balanced) and n=6 (unbalanced). At 119mJ, the between variance was 135 and within variance 670. These values gave a power of 0.80 when n=25 and 0.22 when n=6. At 300mJ, the between variance was 4392 and within variance 1700, resulting in a power of 1 when n=25 and 0.99 when n=6. At the higher UV dose, the power is good, but it would have been desirable to have had a

higher level of power at the lower UV dose. Future studies could achieve this by increasing the sample size.

Erythema induced by incremental doses of UVR by XRCC3 exon 7 genotype in group 2.

UVR on inner forearm, measured at 24 hours, n=31.

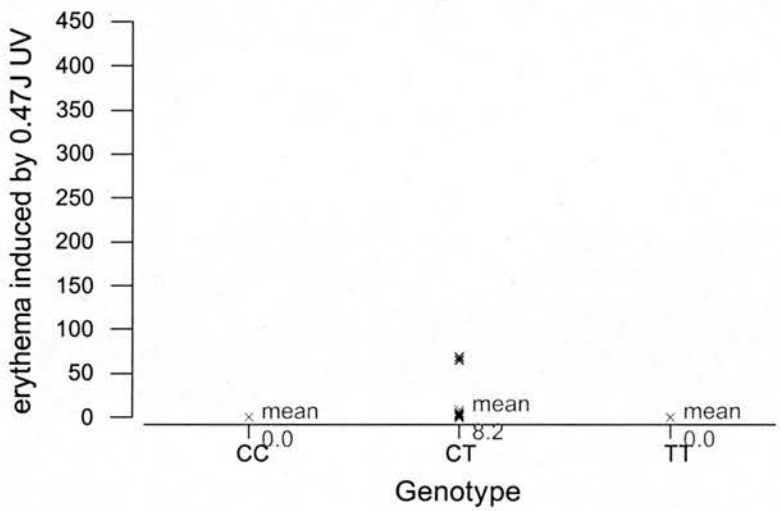


Figure 151. Erythema induced by 0.47J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

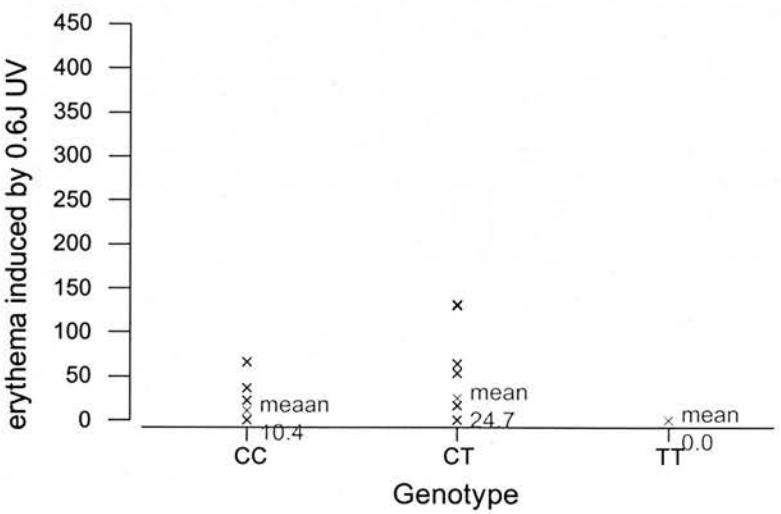


Figure 152 Erythema induced by 0.6J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

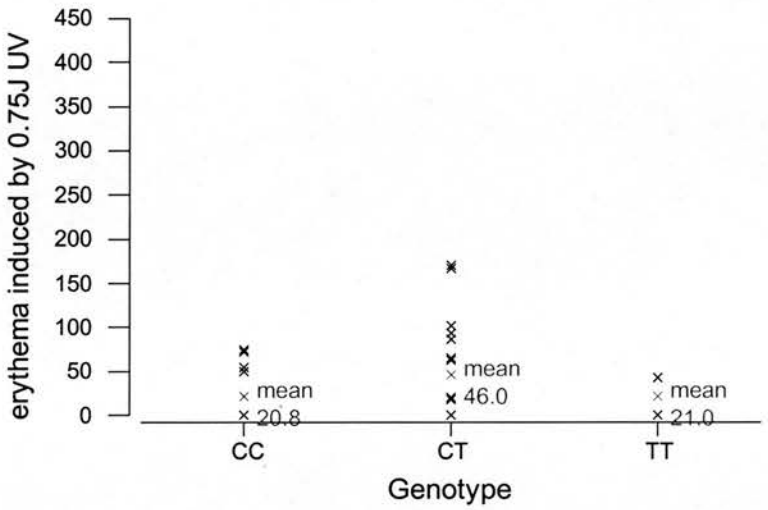


Figure 153 Erythema induced by 0.75J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

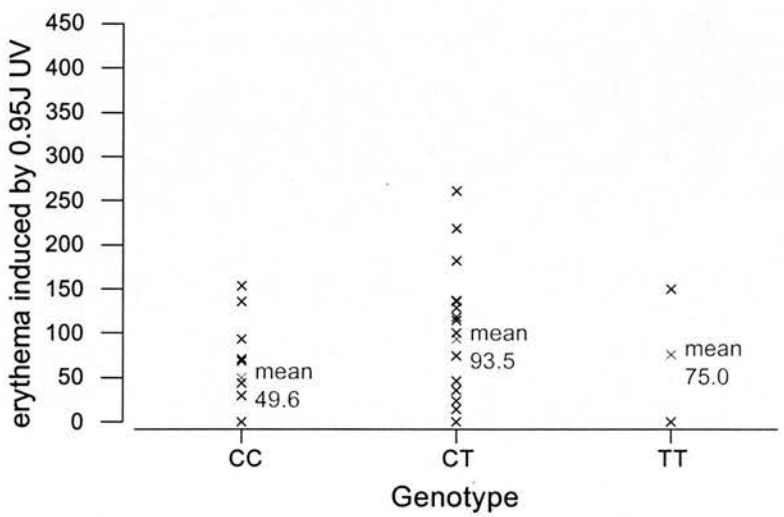


Figure 154 Erythema induced by 0.95J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

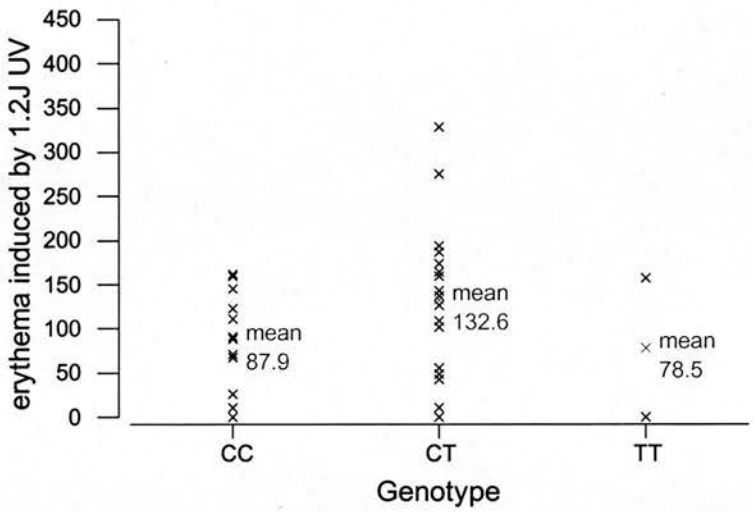


Figure 155 Erythema induced by 1.2J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

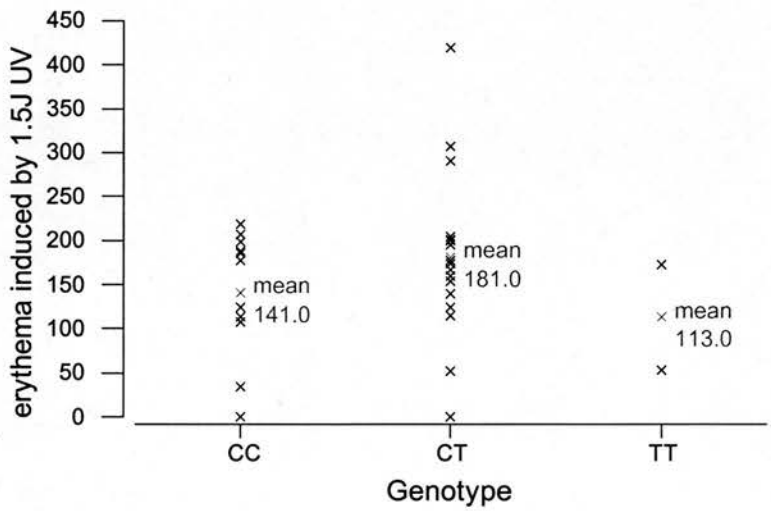


Figure 156. Erythema induced by 1.5J per cm^2 UV by XRCC3 exon 7 genotype.
UVR on inner forearm, measured at 24 hours, n=31.

Analysis of XRCC3 exon 7 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	0.00	0.00	0.00
CT	17	8.24	21.97	5.33
TT	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	521	260	0.94	0.401
Error	28	7725	276		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	10.42	21.12	6.10
CT	17	24.7	44.4	10.8
TT	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.6J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	2107	1053	0.81	0.455
Error	28	36449	1302		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	20.83	31.43	9.07
CT	17	46.0	59.0	14.3
TT	2	21.0	29.7	21.0

Analysis of variance of erythema induced by 0.75J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	4869	2434	1.01	0.377
Error	28	67539	2412		
Total	30	72408			

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	49.6	55.4	16.0
CT	17	93.5	79.4	19.2
TT	2	75.0	106.1	75.0

Analysis of variance of erythema induced by 0.95J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	13539	6770	1.30	0.288
Error	28	145810	5207		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	87.9	55.6	16.1
CT	17	132.6	88.0	21.3
TT	2	78.5	111.0	78.5

Analysis of variance of erythema induced by 1.2J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	16451	8225	1.35	0.275
Error	28	170137	6076		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
CC	12	141.0	69.2	20.0
CT	17	181.0	95.7	23.2
TT	2	113.0	84.9	60.0

Analysis of variance of erythema induced by 1.5J per cm^2 UV

Source	DF	SS	MS	F	P
Factor	2	16181	8090	1.10	0.347
Error	28	206261	7366		
Total	30	222442			

The higher mean level of erythema seen in the TT genotype in group 1 was not observed in group 2. The low number of TT homozygotes could have masked any effect of this

genotype, the CT genotype was highest at all doses. The P values did not approach significance at any dose, ranging from 0.455 to 0.275. Most of the variation can be seen to be due to factors other than genotype.

Power calculations were performed using n=10 and n=2. At 0.47J, the between variance value was 260 and within variance 276. This gave a power of 0.96 when n=10, and 0.18 when n=2. At 1.5J, the between variance value was 8090, and within 7366, power was 0.98 when n=10, and 0.20 when n=2. The true power will therefore lie between a large range, and in order to let the p values be accepted with more confidence, the power would need to be increased at the lower UV doses. This could be achieved by using a larger study group.

No significant association was observed between genotype and erythema levels at any UV dose in either study group. The TT genotype has been previously associated with malignant melanoma in a study by Winsey and colleagues (2000). From this, one would expect the TT genotype to be more sensitive to the effects of UVR, that is having higher levels of erythema in response to UV. This can be observed in the first study group, but is not significant. In group 2, no such observation is seen. A larger study to investigate this polymorphism would be useful, both for determining erythemal response, but also the risk of melanoma and other skin cancers. A study with a large number of melanoma patients, and, importantly, healthy controls with no history of skin cancer would resolve any ambiguity over the possibility that the use of cadavers affected the outcome in the study by Winsey *et al.* A larger study should also allow for a greater number of TT homozygotes, which could resolve the issue of whether erythemal response is associated with this genotype.

The XPG exon 15 polymorphism looked promising as a candidate involved with determining sensitivity to UVR. A non-synonymous change occurs in the amino acid sequence with the presence of this polymorphism, from the basic histamine to the acidic aspartate, which could have a functional effect on the resulting protein. In order to investigate whether the polymorphism had any functional role, an experiment investigating its effect on UV-induced apoptosis in cultured lymphocytes was planned. It was not possible to obtain sufficient fresh blood samples from individuals with each genotype in order to investigate any effect the exon 15 polymorphism might have on UV-induced apoptosis. However, as most is known about UV-induced apoptosis on mouse cells, it was decided to determine whether human lymphocytes undergo apoptosis in a dose-dependent manner.

Lymphocytes were obtained from fresh blood samples, donated by volunteers in the Department of Dermatology. Blood samples were processed as described in Chapter 2, Methods, within one hour of blood donation. Cells were grown in culture for three days, and then irradiated with UVR. Annexin V-FITC and DNA content assays were performed in order to detect levels of apoptosis and cell cycle changes, and analysed by flow cytometry.

Apoptosis is the programmed cell death of cells, where, after the process has been initiated, cells are destroyed. It can be characterised morphologically by condensation of nuclear chromatin, compaction of cytoplasmic organelles, cell shrinkage and changes at the cell surface. At a late stage in the process, the cell in phagocytosed by neighbouring cells.

Annexin V-FITC assay

The Annexin V-FITC conjugates to facilitate rapid fluorimetric detection of apoptotic cells. Early in the apoptotic process, the phospholipid asymmetry of the cytoplasmic membrane of cells is disrupted. This leads to the exposure of the phosphotidyiserine on the outer surface of the cytoplasmic membrane. Annexin V is an anticoagulant protein, which preferentially binds negatively charged phospholipids, therefore, when the

cytoplasmic membrane is disrupted it can bind to the cell surface. The inclusion of propidium iodide in this assay allows the detection of different stages of apoptosis. The flow cytometer was set so that the FL1 channel (X-axis of flow charts) would reflect the log Annexin V-FITC fluorescence, and the FL2 channel reflected the propidium iodine reflectance (Y-axis on flow charts). Cells which bound Annexin V alone were early apoptotic and appear in the lower right quadrant of the flow charts (A4). Cells which take up both Annexin V and PI are late apoptotic, and appear in the upper right quadrant (A2). Cells which are negative for both Annexin V and PI (lower left quadrant, A3) are normal, viable cells.

Initial UV doses were 20 and 40J at a wavelength of 254nm. These were chosen on the basis of past experience in the Melton laboratory, where mouse lymphocytes were shown to display easily detectable levels of UV-induced apoptosis at these UV doses (personal communication with Prof. Melton). UVR was delivered to the cells through the lymphocyte growth medium, as the cultured lymphocytes were not adherent to the dishes and were growing in suspension. This resulted in some of the radiation being filtered out, and the cells therefore did not receive the full dose of UV to which they were exposed. The problem of the media filtering out some UVR could have been circumvented by replacing the growth medium with phosphate buffered saline (PBS). This would involve collecting the cells by centrifugation before resuspending them in PBS, a clear solution that allows UV to pass through it. However, this process of centrifugation and resuspension of cells in PBS to irradiate them, and then again to resuspend them in growing medium afterwards would cause additional, unnecessary, stress to the cells. Exposing the cells to additional stress could induce apoptosis, and it was preferred to keep other sources of apoptosis to a minimum so as not to interfere with any apoptosis induced by UVR.

Effect of 20 and 40J UV on lymphocytes.

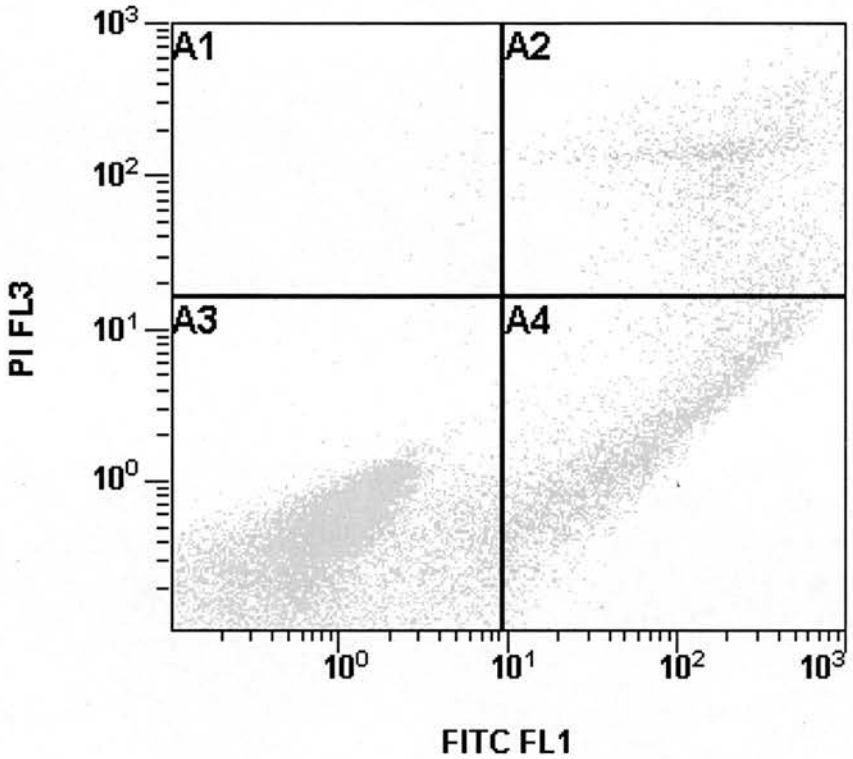


Figure 157 Cells grown in culture for 3 days, control

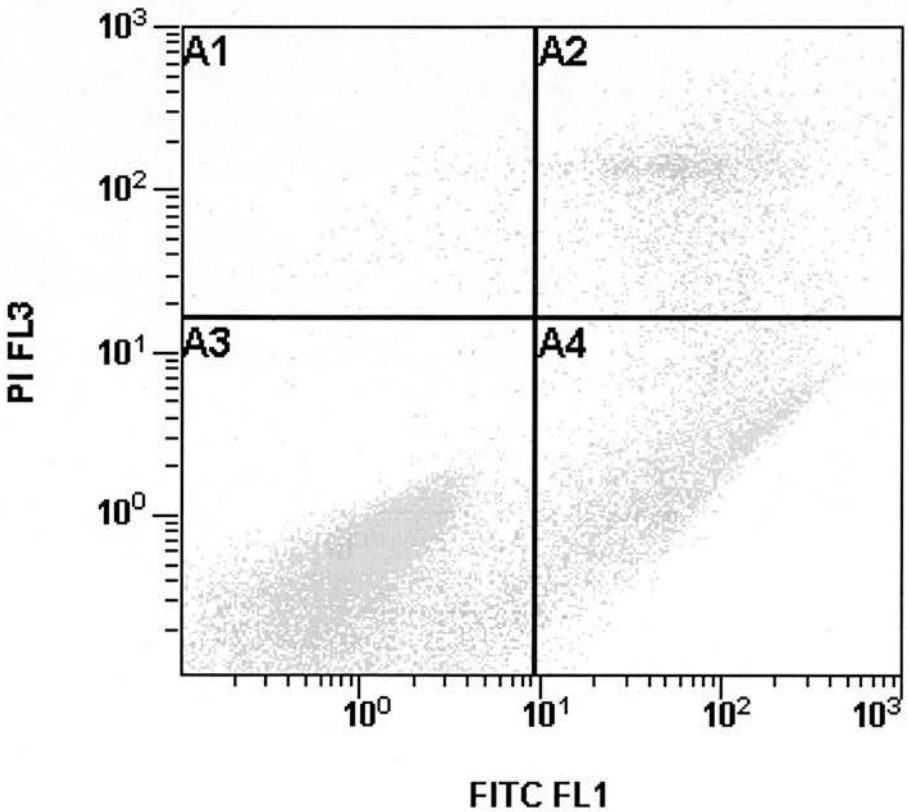


Figure 158 Cells grown in culture for 3 days, irradiated with 20J UV

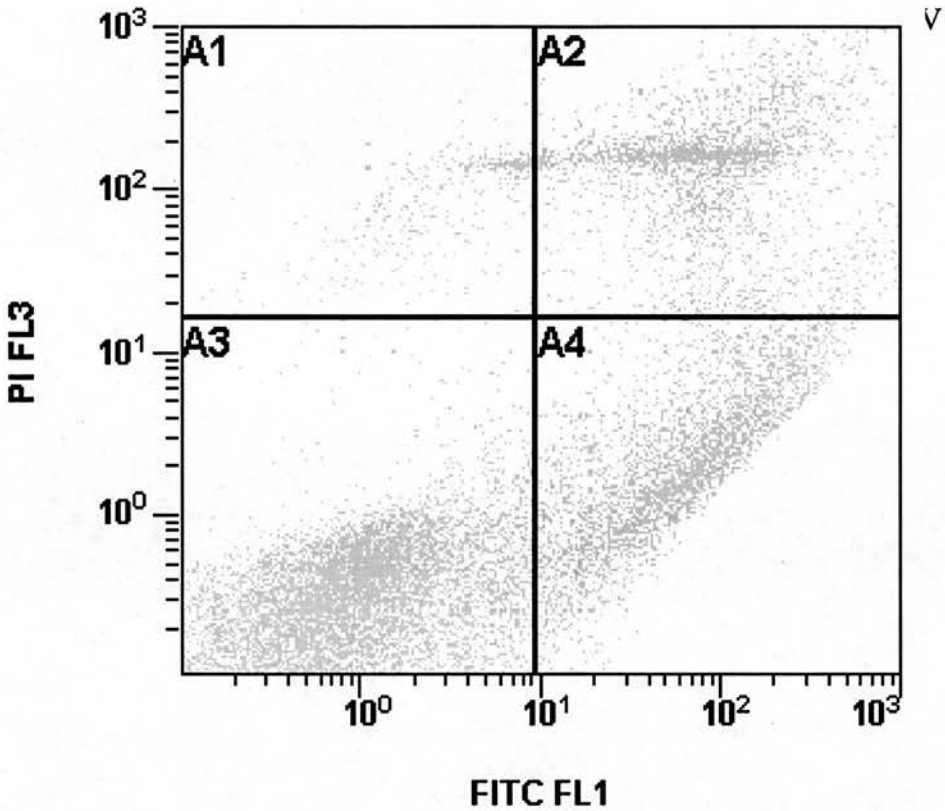


Figure 159 Cells grown in culture for 3 days, irradiated with 40J UV

Percentage of cells in each quadrant.

Quadrant	Control	20J	40J
A1- necrotic cells	0.14%	0.85%	1.93%
A2 – late apoptosis	7.74%	9.81%	14.02%
A3 – healthy cells	75.2%	72.2%	68.34%
A4 –early apoptosis	16.91%	17.35%	15.71%

The initial pilot experiments revealed that little apoptosis was induced following exposure to the nominal UV doses of 20 and 40J. 40J did cause an increased percentage of cells to undergo apoptosis, although this only led to a 7% decrease in the number of healthy cells compared to the control. This was unexpected, as previous studies in the Melton group on mouse lymphocytes had shown these UV doses to be sufficient to induce substantial apoptosis. This protocol was repeated with similar results, and it was concluded that human lymphocyte cells have a much greater resistance to UV-induced apoptosis than their mouse counterparts.

It can also be seen that human lymphocytes undergo a substantial amount of spontaneous apoptosis when grown in culture. Almost 25% of the control population of cells had either started to undergo apoptosis, or had become necrotic.

To investigate whether higher doses of UV could induce greater levels of apoptosis the cells were irradiated with the nominal doses of 100 and 400J in lymphocytes cultured from two different volunteers.

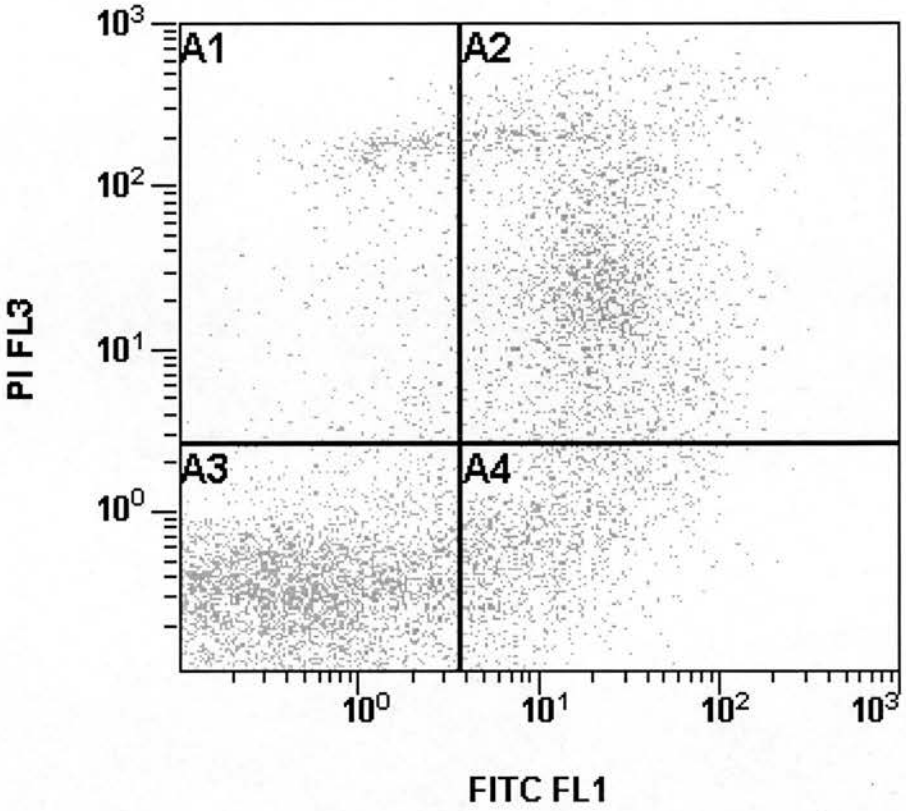


Figure 160 Cells grown in culture for 3 days, no UV

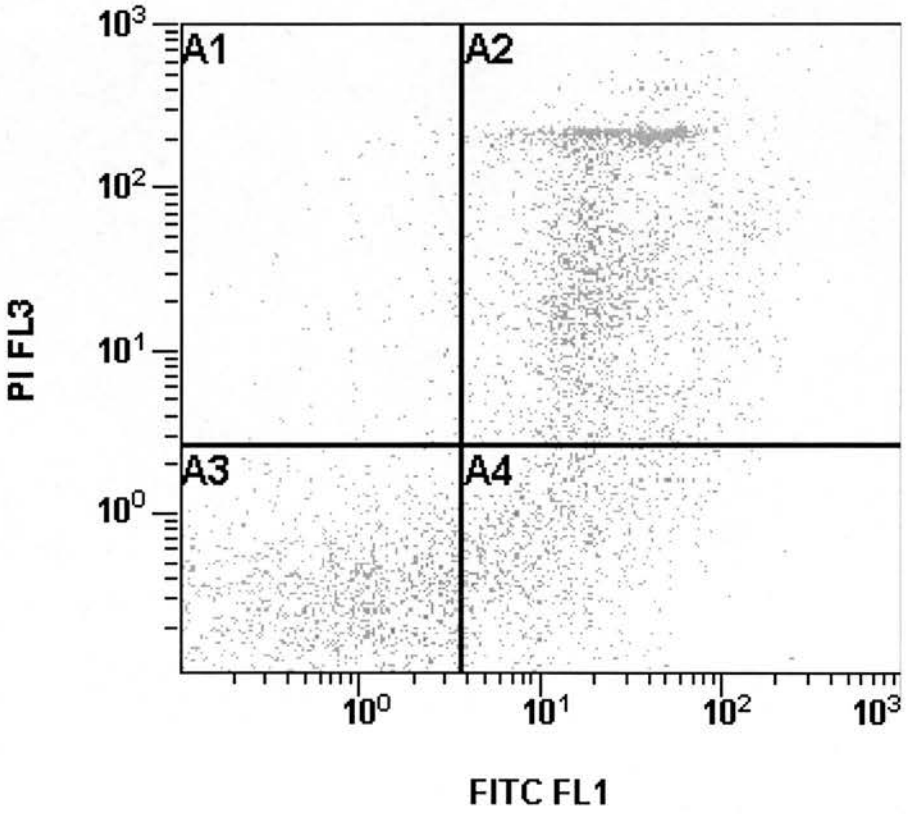


Figure 161 Cells grown in culture for 3 days, irradiated with 100J UV

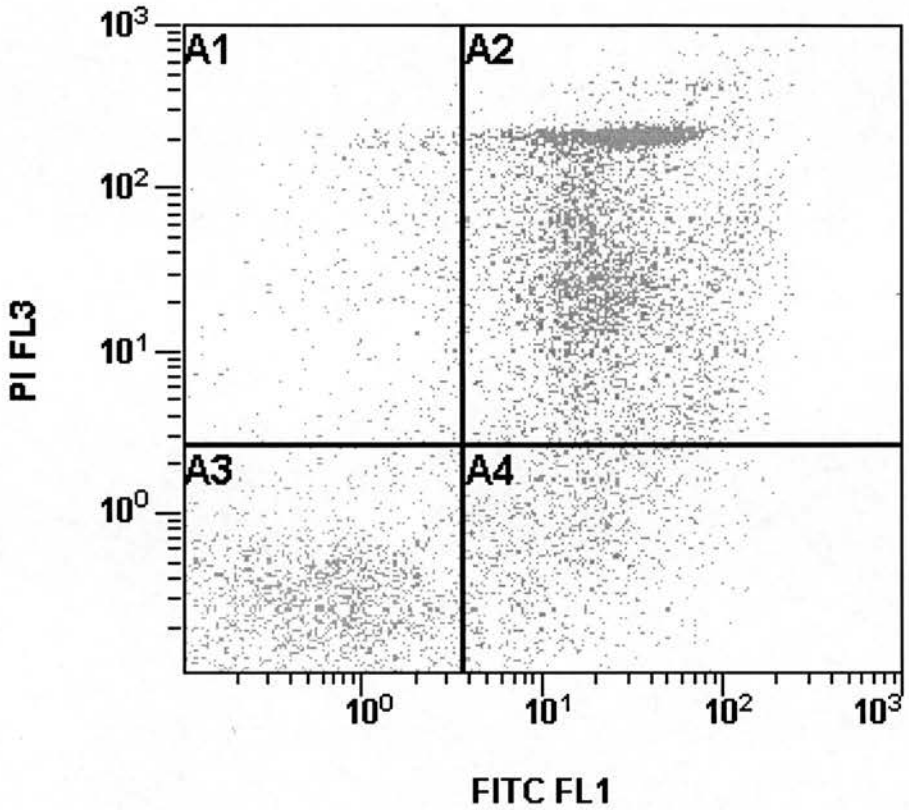


Figure 162 Cells grown in culture for 3 days, irradiated with 400J UV

Quadrant	Control	100J	400J
A1- necrotic cells	4.12%	1.67%	3.61%
A2 – late apoptosis	21.96%	38.55%	51.46%
A3 – healthy cells	60.88%	37.53%	27.83%
A4 –early apoptosis	13.08%	22.25%	17.09%

As can be seen from the flow charts and above table, the higher doses of UV induced much greater levels of apoptosis. The percentage of cells which were healthy and viable decreased in a dose dependent manner, from 60.88% in the control population to 37.53% after 100J and 27.83% after 400J. The high levels of spontaneous apoptosis is again seen in the control population, with 4.12% necrotic. The percentage of necrotic cells is less in the two populations which have been irradiated, but here most cells are in early or late apoptosis. A shift from early to late apoptosis can be seen as the UV dose increases, indicating that not only does the higher UV dose induce more cells to undergo apoptosis, these cells also do so faster.

Cells from donor 2. Effect of 100 and 400J UV on lymphocytes

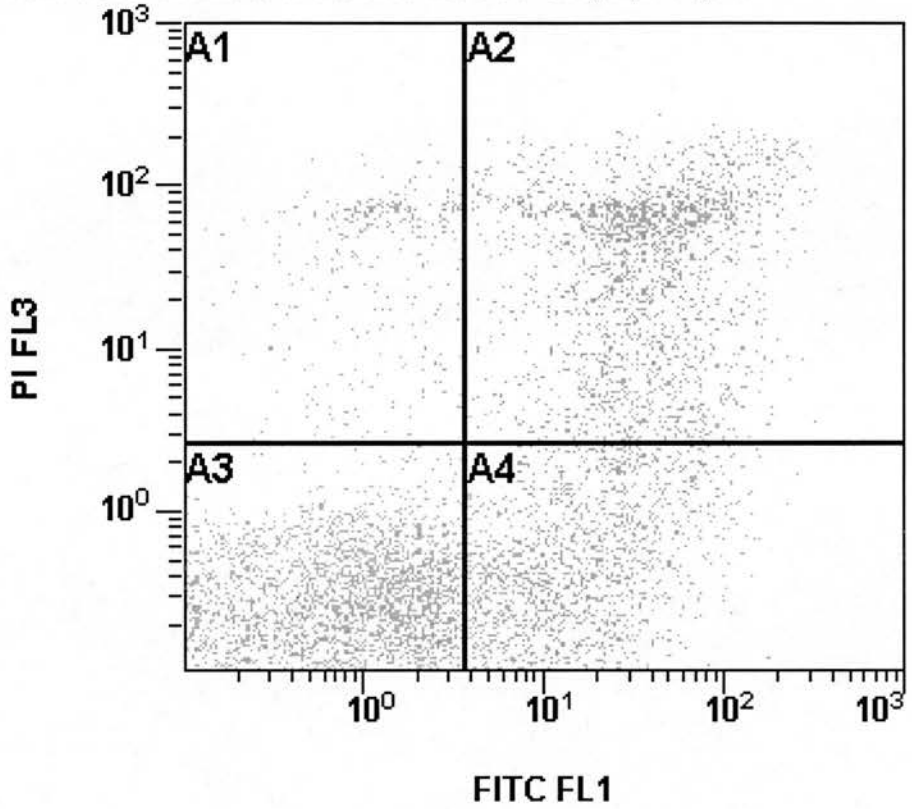


Figure 163 Cells grown in culture for 3 days, no UV

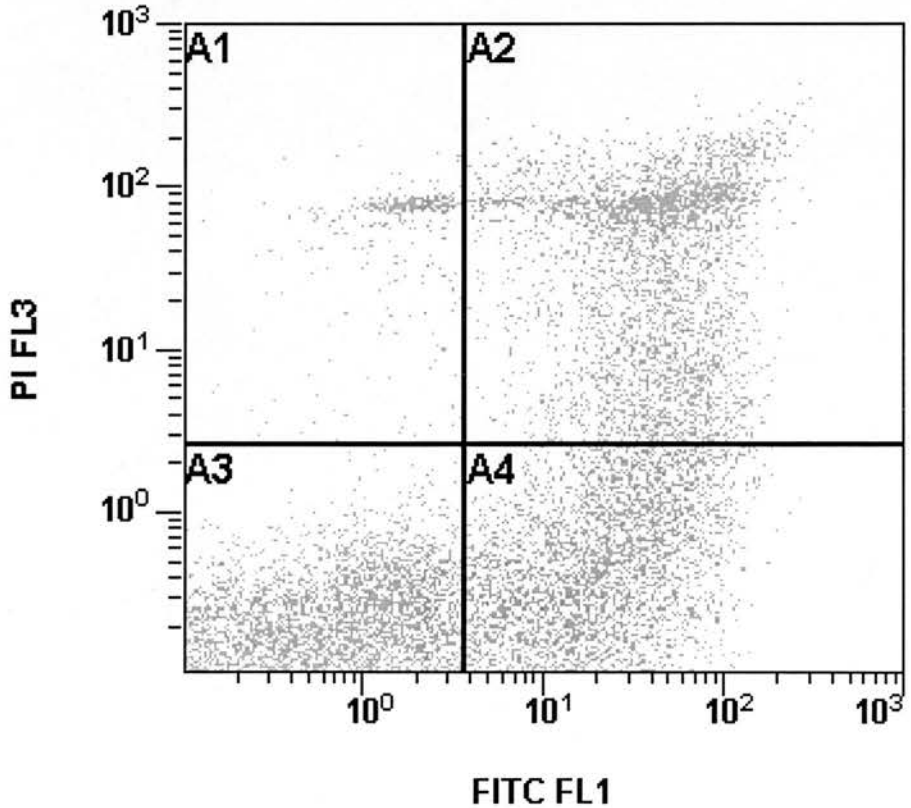


Figure 164 Cells grown in culture for 3 days, irradiated with 100J UV

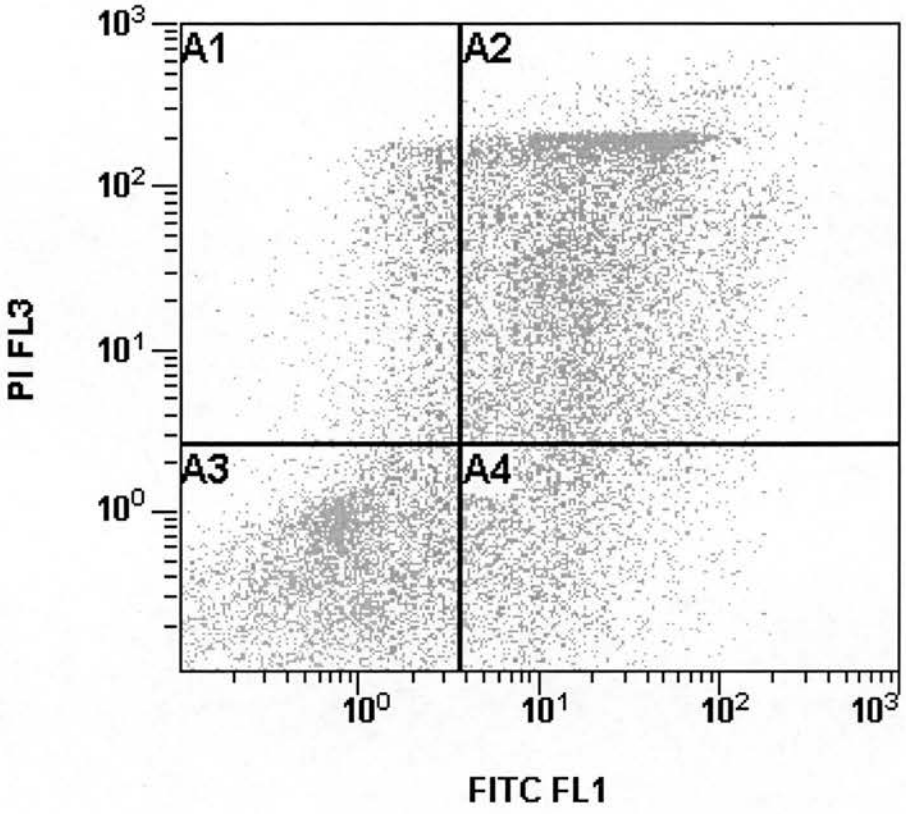


Figure 165 Cells grown in culture for 3 days, irradiated with 400J

Quadrant	Control	100J	400J
A1- necrotic cells	2.67%	1.88%	8.38%
A2 – late apoptosis	15.95%	17.84%	42.79%
A3 – healthy cells	58.52%	43.95%	29.57%
A4 –early apoptosis	22.86%	36.33%	19.26%

In the cells cultured from donor 2, there was again dose dependent UV-induced apoptosis. The percentage of healthy cells decreased, from 58.22% in the control population, to 43.95% after 100J UV, and 29.57% after irradiation with 400J UV. There was a more dramatic shift from early to late apoptosis as the UV dose increased than seen in the cells from donor 1.17.84% of the cells irradiated with 100J were late apoptotic, while after 400J 42.79% were in the late stage of apoptosis. This is illustrated in the shift of cells from quadrant A4 to A2 in the flow charts above, demonstrating again that higher doses of UV both induce greater levels of UV, and do so faster. This suggests that the higher UV dose is inducing a greater amount of DNA damage than 100J. The repair pathways will be less able to cope with the greater amount of damage, thus the apoptosis pathways will be triggered so as the UV-induced mutations are not incorporated into the genome.

DNA content

Flow cytometry was also used to measure DNA, to examine the effect of UVR on the cell cycle. Cells start the cell cycle in G1, before entering S-phase (where DNA synthesis occurs). DNA content increases, until it doubles and the cell has entered G2. Cells then enter mitosis (M-phase), and divide, before beginning the cell cycle again. Cells which are not currently cycling are in the G0 phase. The different stages of cell cycle can be identified in a DNA histogram, as seen in the diagram below.

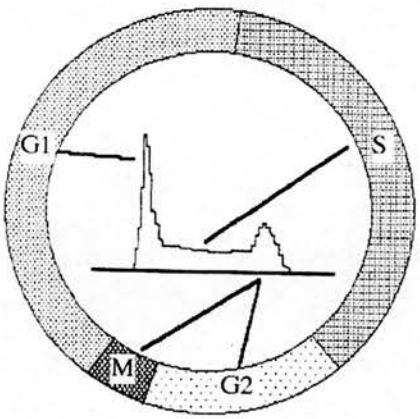


Figure 165. The cell cycle and DNA content of the cell. (Adapted from Darzynkiewicz *et al*, 1992).

Propidium iodide can be used to determine the viability of cells, as it is excluded by viable cells, and binds to nucleic acids only in dead or dying cells, where it fluoresces red.

At a late stage in apoptosis, double-strand breaks are introduced into the DNA at the linker regions between the nucleosomes. Some of the lower molecular weight fragments are lost during fixation of the cells, which leads to a lowering of the DNA content. On a DNA histogram these cells can be seen as a sub-G1 peak (Darzynkiewicz *et al*, 1992).

DNA content

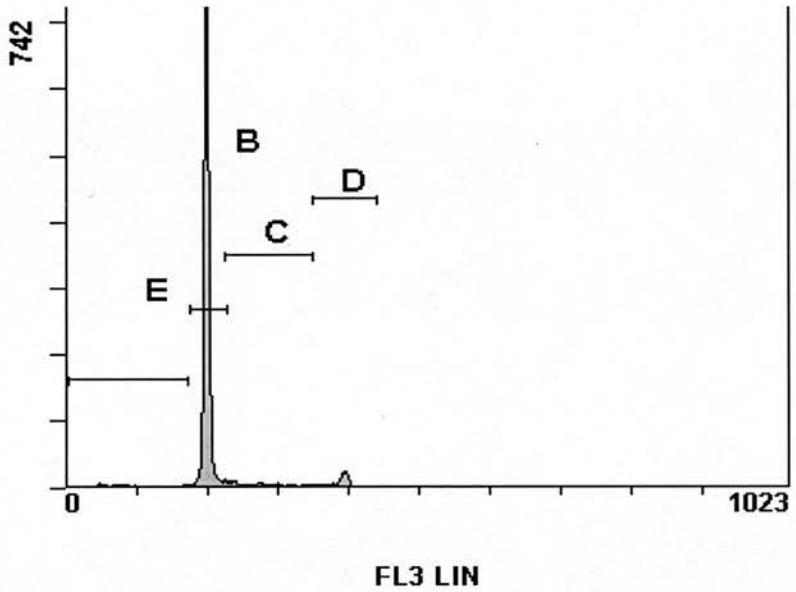


Figure 166 Cells grown in culture for 3 days, no UV

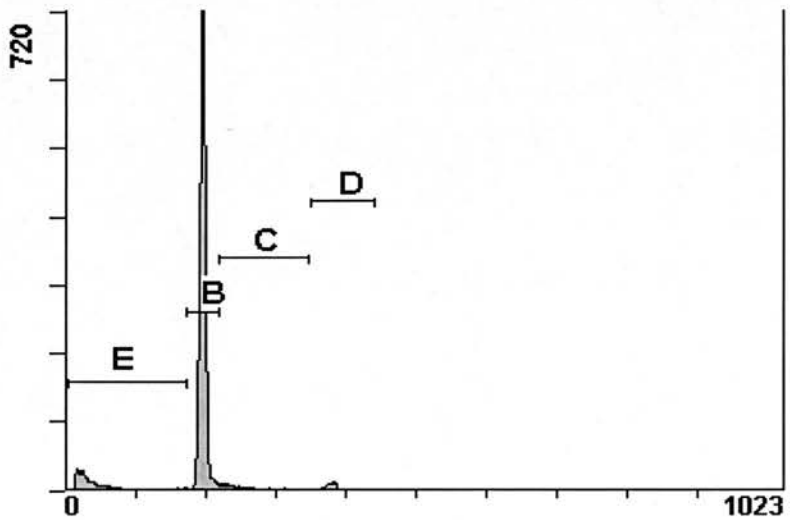


Figure 167 Cells grown in culture for 3 days, 100J UV

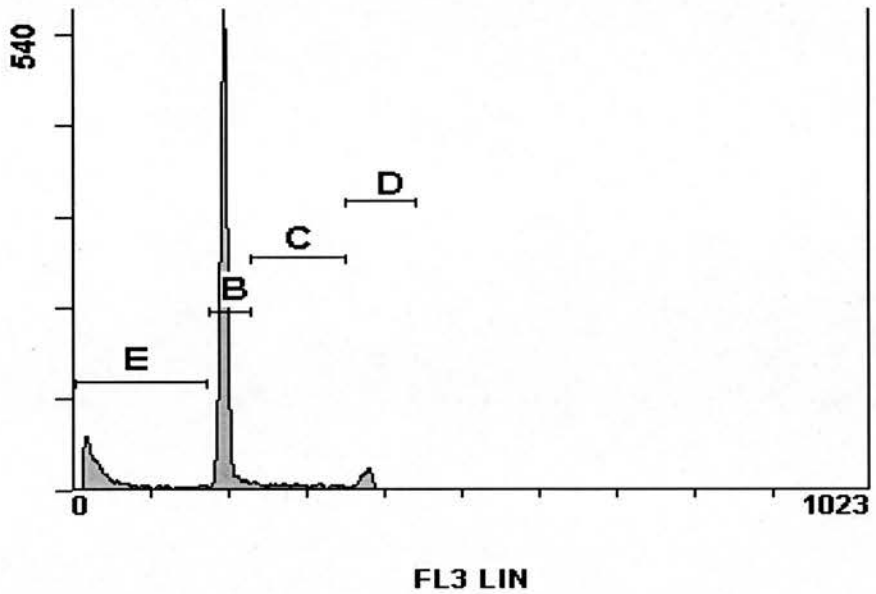


Figure168 Cells grown in culture for 3 days, 400J UV

DNA content of cells, percentage of population in each peak

Peak	Control	100J	400J
E	5.85%	10.99%	16.39%
B	84.13%	80.93%	70.69%
C and D	10.02%	8.08%	12.92%

Cells from donor 4. DNA content assay, 100J and 400J UV

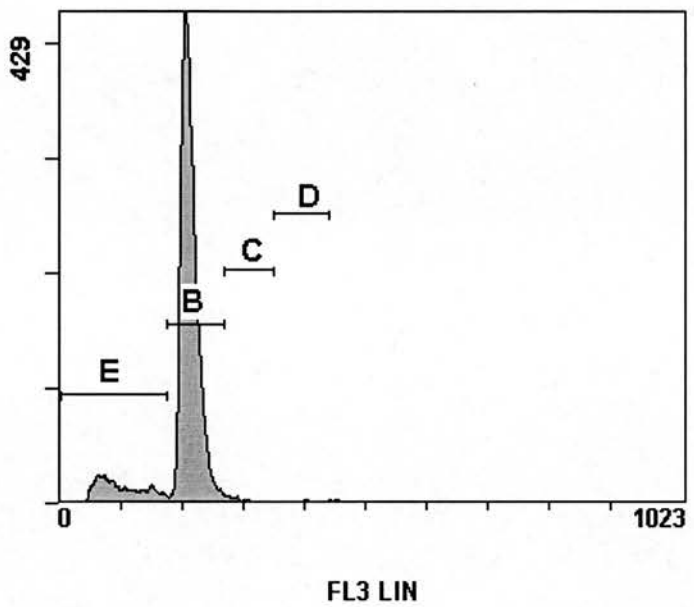


Figure 169 Cells grown in culture for 3 days, no UV

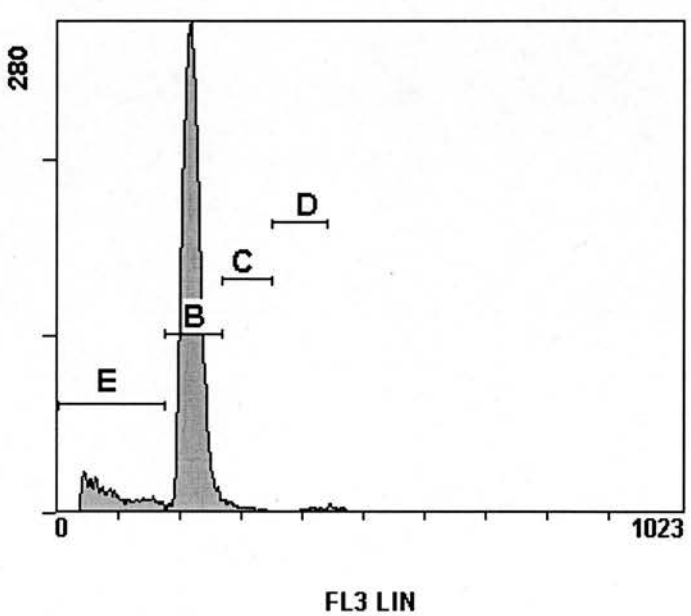


Figure 170 Cells grown in culture for 3 days, 100J UV

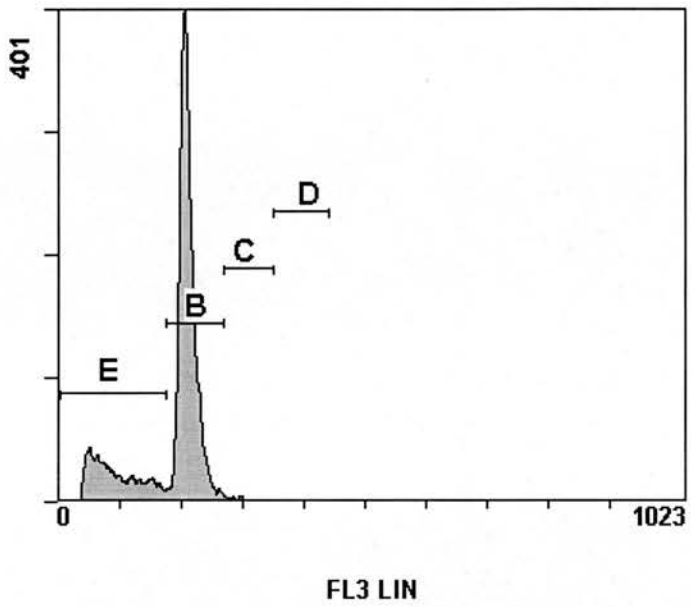


Figure 171 Cells grown in culture for 3 days, 400J UV

DNA content of cells, percentage of population in each peak

Peak	Control	100J	400J
E	13.3%	13.51%	23.52%
B	81.72%	79.35%	72.48%
C and D	4.98%	7.4%	4%

The DNA content assay was used to determine what proportion of cells were undergoing mitosis and replicating their DNA content. The E peak seen on the flow charts above corresponds to cells which are sub-G1 phase of the cell cycle. These cells are not undergoing mitosis, and no DNA replication is occurring in them. It is this proportion that are of interest, as these cells have damaged DNA and are not undergoing mitosis to prevent the mutations they have acquired being incorporated into the genome.

In both cells samples which were analysed for DNA content, there is a dose dependent increase in the number of cells that are sub-G1, and a decrease in the number of healthy, viable cells. This indicates that there is a dose dependent increase in DNA damage occurring within the cells.

Discussion

From this experiment, it can be seen that UVR induces DNA damage in a dose dependent manner in cultured human lymphocytes. There is a related dose dependent increase in the levels of UV-induced apoptosis. Cultured human lymphocytes display fairly high levels of spontaneous apoptosis, as seen from the control data. There are differences in the levels of apoptosis induced by UV between individuals.

Chapter 5 Analysis of repair gene polymorphisms for association with response to anthralin.

As previously mentioned, anthralin is an irritant to the skin, and induces oxidative stress, which leads to DNA damage in the same way that UVR induces DNA damage indirectly through the generation of reactive oxygen species. Such DNA damage is repaired in the main by the BER pathway, but also by the NER pathway. Therefore, if any of the polymorphisms investigated here were to be associated with cutaneous sensitivity following exposure to anthralin, one would expect this to be the XRCC1 exon 10 polymorphism, XRCC1 being crucial for efficient BER.

Sensitivity to anthralin was measured by laser Doppler flow cytometry. This technique measures blood flow in the superficial dermis. The blood flow measured by the laser Doppler technique is referred to as "flux", a quantity proportional to the product of the average speed of the blood cells and the blood volume, and is measured in arbitrary units (Farr and Diffey; 1986). Upon application of an irritant, such as anthralin, a feature of the immune response is vasodilation of blood vessels in the superficial dermis and an increase in blood flow to the surface of the skin. The greater the sensitivity to an irritant, the greater the blood flow, therefore a higher level of "flux" response to anthralin would indicate increased sensitivity to the substance.

XPD exon 6

Flux response to increasing doses of anthralin by XPD exon 6 genotype in group 1.

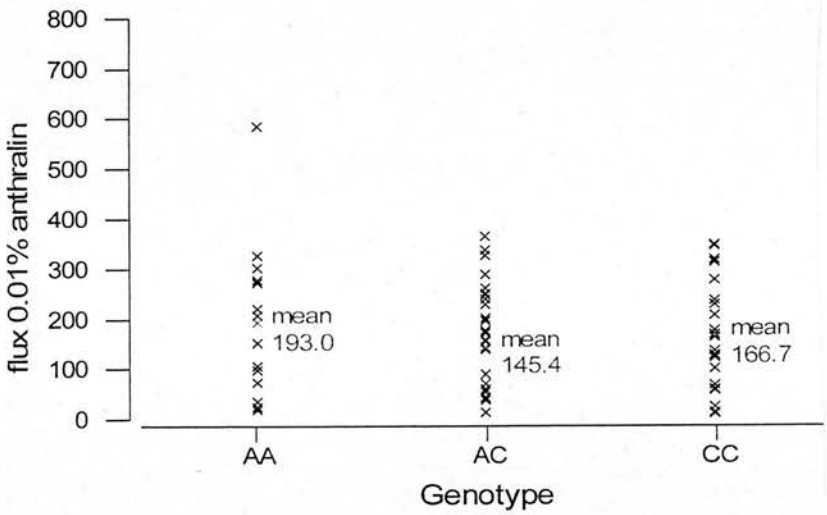


Figure 172. Flux induced by 0.01% Anthralin by XPD exon 6 genotype.
Anthralin on lower back, flux measured at 48 hours

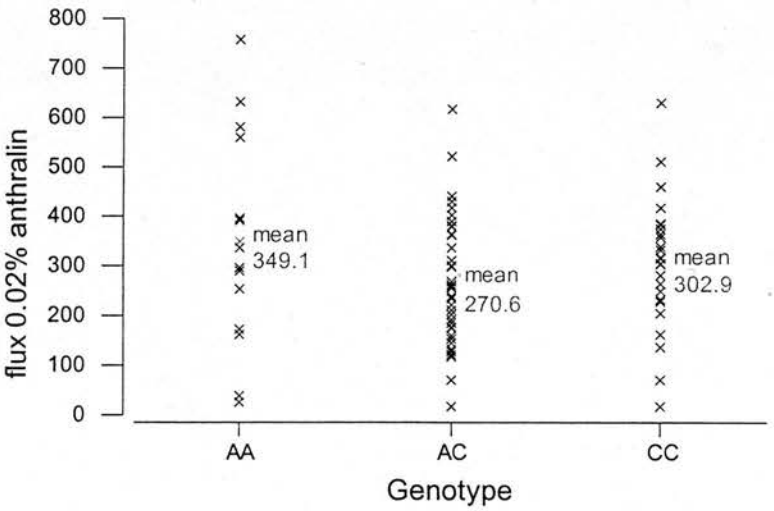


Figure 173 Flux induced by 0.02% Anthralin by XPD exon 6 genotype.
Anthralin on lower back, flux measured at 48 hours

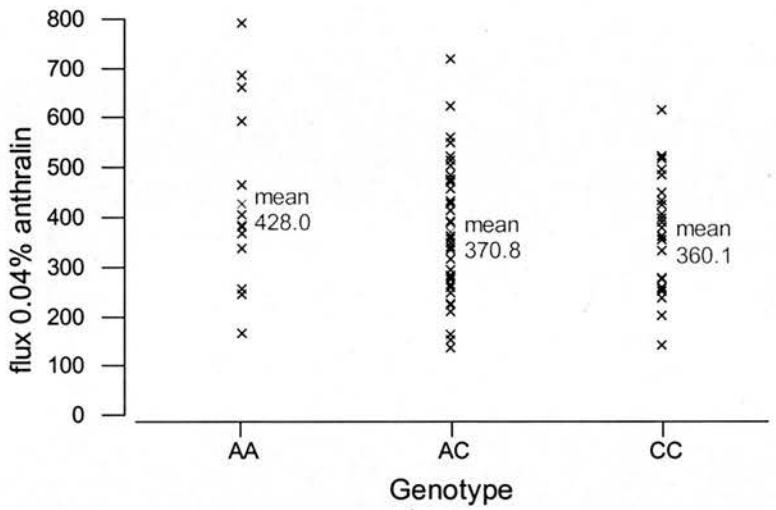


Figure 174 Flux induced by 0.04% Anthralin by XPD exon 6 genotype.
Anthralin on lower back, flux measured at 48 hours

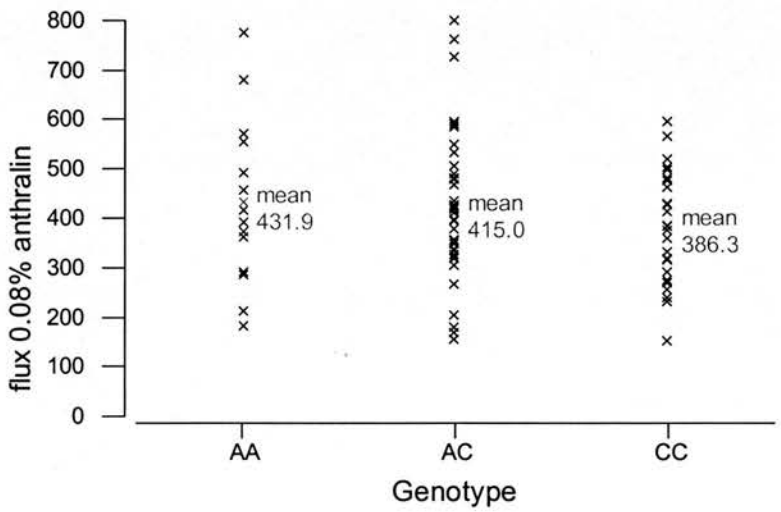


Figure 175 Flux induced by 0.08% Anthralin by XPD exon 6 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux induced by anthralin and XPD exon 6 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	14	193.0	155.5	41.6
AC	39	145.4	104.2	16.7
CC	25	166.7	111.6	22.3

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	24560	12280	0.90	0.412
Error	75	1025767	13677		
Total	77	1050326			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	14	349.1	220.6	59.0
AC	39	270.6	128.8	20.6
CC	25	302.9	133.5	26.7

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	65804	32902	1.46	0.239
Error	75	1690984	22546		
Total	77	1756788			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	14	428.0	188.4	50.4
AC	39	370.8	134.5	21.5
CC	25	360.1	116.1	23.2

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	44989	22495	1.15	0.323
Error	75	1471768	19624		
Total	77	1516757			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	14	431.9	171.1	45.7
AC	39	415.0	152.4	24.4
CC	25	386.3	115.8	23.2

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	21655	10828	0.51	0.601
Error	75	1585610	21141		
Total	77	1607265			

At no dose of anthralin examined was there an XPD exon 6 genotype-dependent response to anthralin. The AA genotype had a higher mean flux in response to anthralin at each dose of anthralin examined, although the standard errors of mean were largest in this group at all doses. P values showed no significant associated between the exon 6 polymorphism and flux, ranging from 0.239 to 0.60. The genotype of individuals explained very little of the variation in response to anthralin, the SS(factor) values being greatly smaller than the SS(error), the two values making up the SS(total), accounting for the total amount of variation observed between individual responses to anthralin. This implies that the exon 6 polymorphism of XPD is not involved with the repair of DNA damage induced by anthralin, which is primarily due to oxidative stress.
Power calculations were carried out for 0.01% and 0.08% using n=26 (balanced) and n=14. At 0.01% anthralin, the between variance value was 12280, and the within variance 13677. This gave a power of 0.99 when n=26, and 0.99 when n=14. 0.08% had a between variance value of 10828 and a within variance value of 21141, giving a power of 0.99 when n=26 and 0.91 when n=4. The true power will lie between these values, but this range is of adequate power.

XPD exon 22

Flux response to increasing doses of anthralin by XPD exon 22 genotype in group 1.

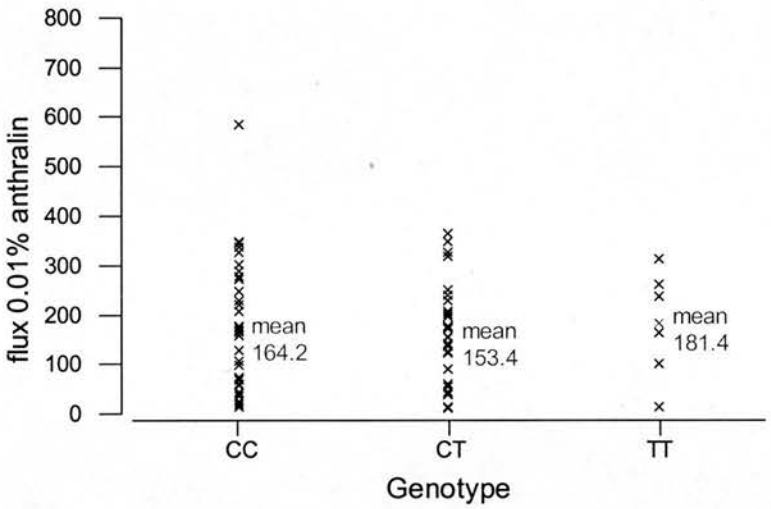


Figure 176 Flux induced by 0.01% Anthralin by XPD exon 22 genotype.
Anthralin on lower back, flux measured at 48 hours

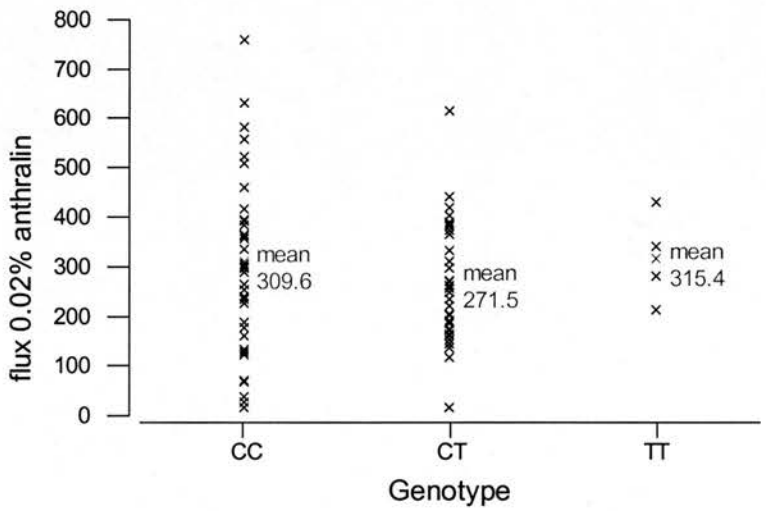


Figure 177 Flux induced by 0.02% Anthralin by XPD exon 22 genotype.
Anthralin on lower back, flux measured at 48 hours

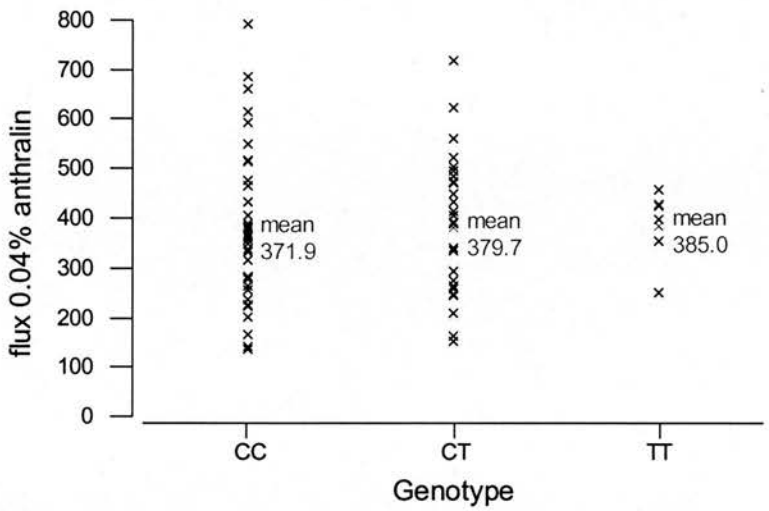


Figure 178. Flux induced by 0.04% Anthralin by XPD exon 22 genotype.

Anthralin on lower back, flux measured at 48 hours

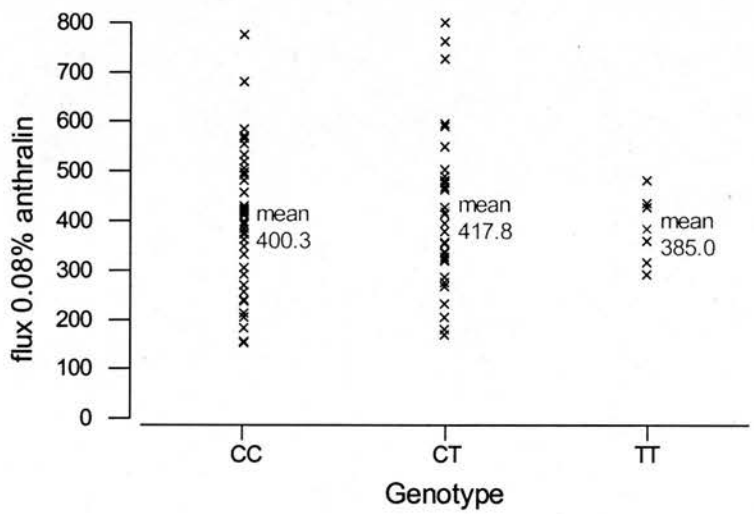


Figure 179 Flux induced by 0.08% Anthralin by XPD exon 22 genotype.

Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and XPD exon 22 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	39	164.2	130.4	20.9
CT	34	153.4	101.2	17.4
TT	6	181.4	111.1	45.3

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	4849	2425	0.18	0.839
Error	76	1045511	13757		
Total	78	1050360			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	39	309.7	179.8	28.8
CT	34	271.5	121.1	20.8
TT	6	315.4	71.8	29.3

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	29486	14743	0.64	0.528
Error	76	1738542	22876		
Total	78	1768028			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	39	371.9	153.9	24.6
CT	34	379.7	134.8	23.1
TT	6	385.0	74.7	30.5

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1596	798	0.04	0.961
Error	76	1527766	20102		
Total	78	1529361			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	39	400.3	142.6	22.8
CT	34	417.8	158.3	27.2
TT	6	385.0	75.3	30.7

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8615	4307	0.20	0.818
Error	76	1628259	21424		
Total	78	1636874			

As with the exon 6 polymorphisms, no association was observed between flux response and the XPD exon 22 genotype. No consistent pattern emerged for levels of erythema between genotypes at different anthralin doses, for example, the heterozygous CT displayed the lowest mean response at 0.01% and 0.02%, but the homozygous CC was the lowest at the highest doses. The TT genotype was the highest at 0.01%, 0.02% and 0.04%, but lowest at 0.08%. The p values reflected this, showing no significance and ranged from 0.961 to 0.528. Analysis of factor and error values shows that almost all the variation was due to something other than genotype. From this data, it can be seen that the XPD exon 22 is not involved with the response to anthralin.

Power calculations were carried out using n=26 and n=6. 0.01% anthralin had a between variance value of 2425 and a within variance value of 13757. This gave a power of 0.76 when n=26, and 0.20 when n=6. 0.08% had a between variance of 4307, and within variance of 21424. This resulted in a power of 0.82m when n=26, and a power of 0.22

when n=6. For this polymorphism, an increase in power would be advantageous, which could be carried out in a future study by using a larger study group.

XPD exon 23

Flux response to increasing doses of anthralin by XPD exon 23 genotype in group 1.

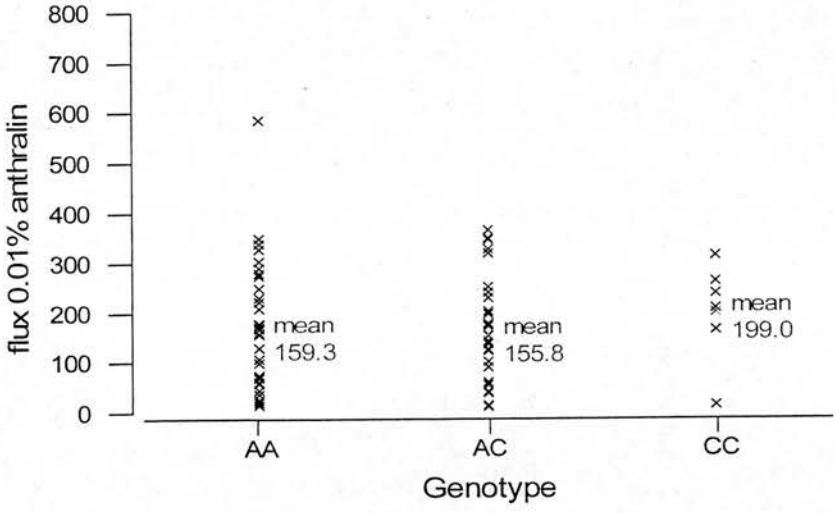


Figure 180 Flux induced by 0.01% Anthralin by XPD exon 23 genotype.
Anthralin on lower back, flux measured at 48 hours

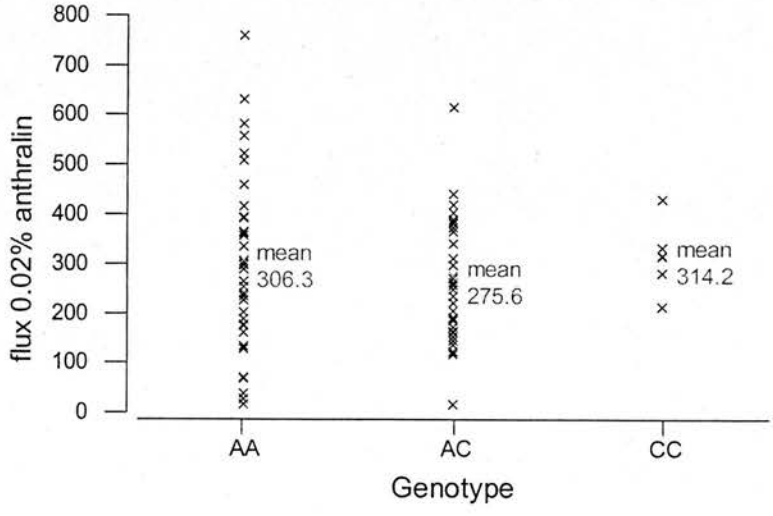


Figure 181. Flux induced by 0.02% Anthralin by XPD exon 23 genotype.
Anthralin on lower back, flux measured at 48 hours

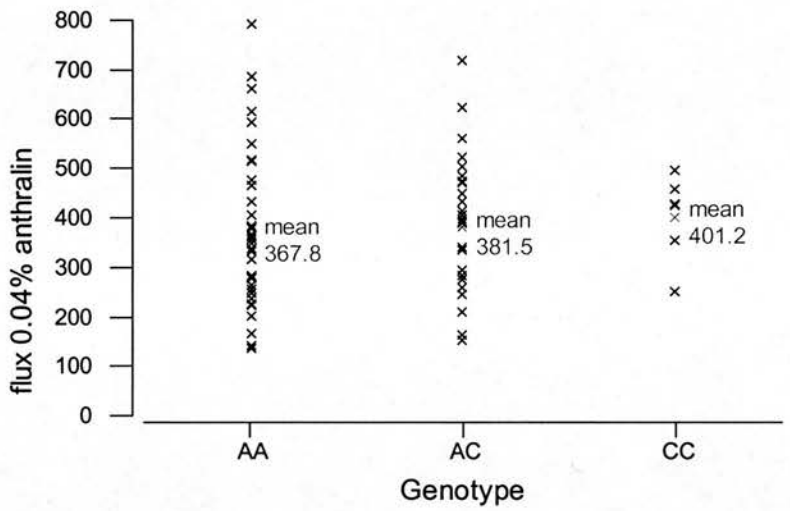


Figure 182 Flux induced by 0.04% Anthralin by XPD exon 23 genotype.

Anthralin on lower back, flux measured at 48 hours

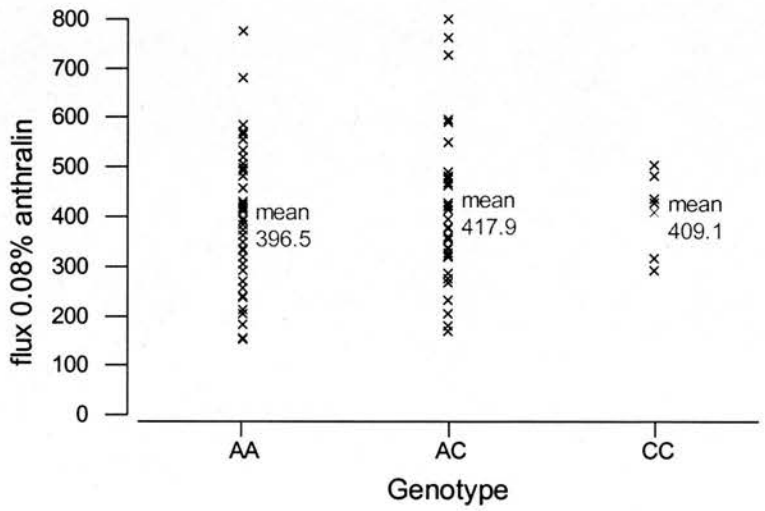


Figure 183 Flux induced by 0.08% Anthralin by XPD exon 23 genotype.

Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and XPD exon 23 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	39	159.4	126.9	20.3
AC	34	155.8	106.5	18.3
CC	6	199.0	103.7	42.3

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	9686	4843	0.35	0.703
Error	76	1040674	13693		
Total	78	1050360			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	39	306.3	179.0	28.7
AC	34	275.6	123.8	21.2
CC	6	314.2	71.4	29.1

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	19848	9924	0.43	0.651
Error	76	1748180	23002		
Total	78	1768028			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	39	367.8	155.5	24.9
AC	34	381.5	130.9	22.4
CC	6	401.2	87.4	35.7

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7445	3723	0.19	0.831
Error	76	1521916	20025		
Total	78	1529361			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
AA	39	396.5	143.5	23.0
AC	34	417.9	156.5	26.8
CC	6	409.1	87.1	35.6

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8333	4166	0.19	0.824
Error	76	1628541	21428		
Total	78	1636874			

Analysis of variance of levels of flux induced by incremental doses of anthralin revealed no significant variation in flux between XPD exon 23 genotypes at any dose examined. P values did not approach the level of significance (p=0.05) at any dose, from p=0.703 at 0.01% anthralin to p=0.824 at 0.08% anthralin. Variation in flux could not be attributed to genotype, with the SS(factor) values being very low compared with the SS(error) values. From this it can be implied that exon 23 is not involved with the repair of anthralin induced DNA damage.

Power calculations were carried out using n=26 (balanced) and n=6 (least balanced). At 0.01%, the between variance value was 4843 and the within variance value 13693. Power was 0.97 when n=26 and 0.37 when n=6. 0.08% anthralin gave a between variance value of 4166 and a within value of 24128. Power was 0.80 when n=26, and 0.22 when n=6. In order to accept the p values with more confidence, the power values would need to be increased.

At none of the three polymorphisms of XPD examined, exon 6, exon 22 and exon 23, was there any association observed between genotype and flux response to anthralin, suggesting that XPD is not involved with the repair of anthralin induced DNA damage, which is mainly due to oxidative stress. The finding of no association between any of the polymorphisms of XPD analysed here and anthralin response is in agreement with the observation that the BER pathway is the main pathway for the repair of oxidative damage.

CKM exon 8

Flux response to increasing doses of anthralin by CKM exon 8 genotype in group 1.

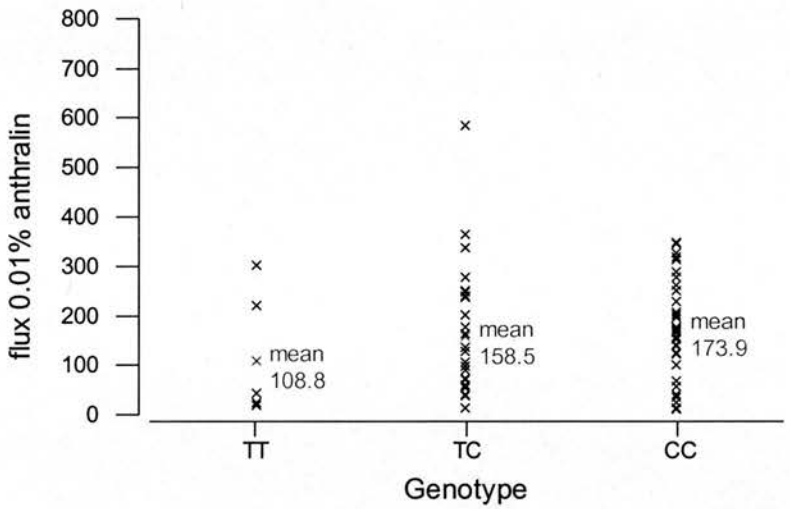


Figure 184 Flux induced by 0.01% Anthralin by CKM exon 8 genotype.
Anthralin on lower back, flux measured at 48 hours

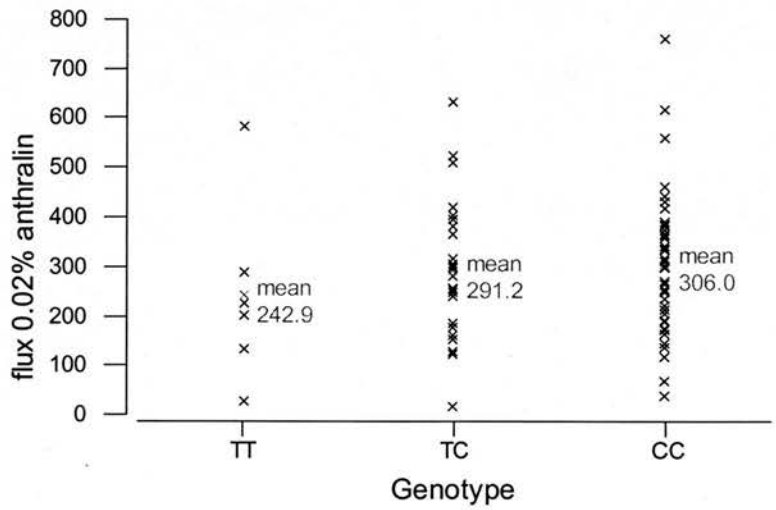


Figure 185 Flux induced by 0.02% Anthralin by CKM exon 8 genotype.
Anthralin on lower back, flux measured at 48 hours

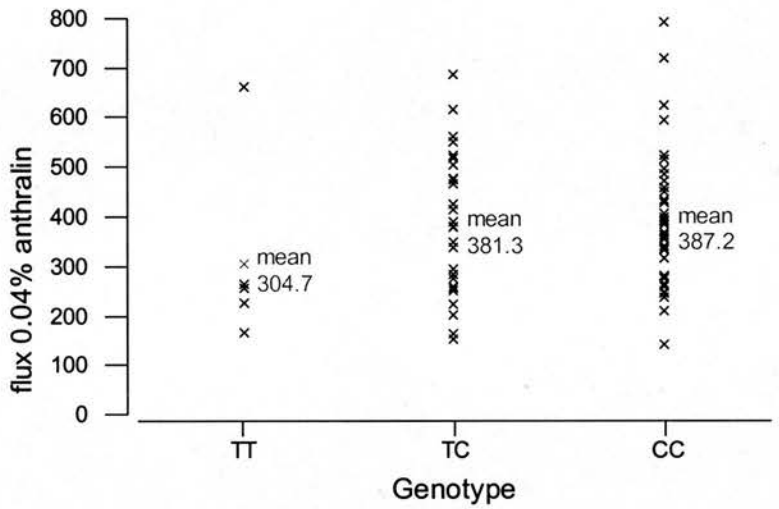


Figure 186 Flux induced by 0.04% Anthralin by CKM exon 8 genotype.
Anthralin on lower back, flux measured at 48 hours

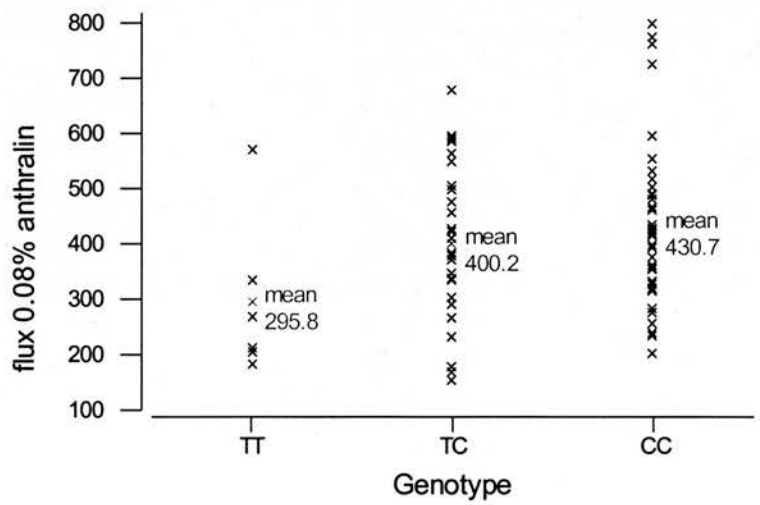


Figure 187 Flux induced by 0.08% Anthralin by CKM exon 8 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and CKM exon 8 genotype in group 1.

0.01% anthralin

Genotype	N	Mean	StDev	SE Mean
TT	6	108.8	120.8	49.3
TC	27	158.5	130.2	25.1
CC	43	173.9	104.3	15.9

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	23257	11629	0.87	0.421
Error	73	970634	13296		
Total	75	993892			

0.02% anthralin

Genotype	N	Mean	StDev	SE Mean
TT	6	242.9	188.6	77.0
TC	27	291.2	161.3	31.0
CC	43	306.0	140.7	21.5

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	21858	10929	0.47	0.625
Error	73	1685032	23083		
Total	75	1706889			

0.04% anthralin

Genotype	N	Mean	StDev	SE Mean
TT	6	304.8	178.6	72.9
TC	27	381.3	143.7	27.6
CC	43	387.2	132.3	20.2

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	36090	18045	0.92	0.403
Error	73	1431802	19614		
Total	75	146789			

0.08% anthralin

Genotype	N	Mean	StDev	SE Mean
TT	6	295.8	145.9	59.6
TC	27	400.2	141.9	27.3
CC	43	430.7	141.3	21.5

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	99159	49579	2.46	0.092
Error	73	1468497	20116		
Total	75	1567656			

No significant association was observed between flux from any anthralin dose and CKM exon 8 genotype. P values ranged from 0.625 to 0.092. Although at 0.08% the p value was lowest, tending towards significance, this could have been influenced by the low number of TT homozygotes, the genotype group with the lowest flux response. Analysis of the factor and error values confirm that variation in response due to genotype contributes towards almost none of the total variation, implying that the vast majority of variation in flux response is due to factors other than genotype, such as chance. This is in agreement with the data observed from the XPD polymorphisms, as CKM was only included as a marker for this repair gene, it itself having no role in repair. Indeed, it would be rather worrying if a significant association had been observed between CKM and flux response, but not in any of the XPD polymorphisms.

N=25 (balanced) and n=6 (least balanced) were used to perform power calculations. 0.01% anthralin had between variance values of 11629 while within variance was 13296. These values gave a power of 0.99 when n=25 and 0.75 when n=6. 0.08% had a between

variance value of 49579, and a within variance values of 20116. The power was 1 both when n=25 and when n=6. Sufficient power was achieved when analysing this polymorphism to be confident that there was no associated between the CKM 8 genotype and flux response to anthralin, as indicated by ANOVA p values.

ERCC1 exon 4

Flux response to increasing doses of anthralin by ERCC1 exon 4 genotype in group 1.

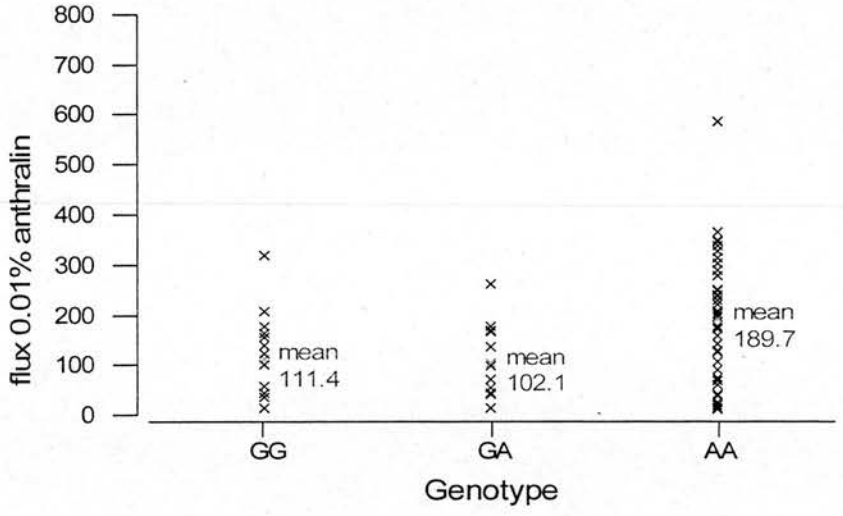


Figure 188. Flux induced by 0.01% Anthralin by ERCC1 exon 4 genotype.
Anthralin on lower back, flux measured at 48 hours

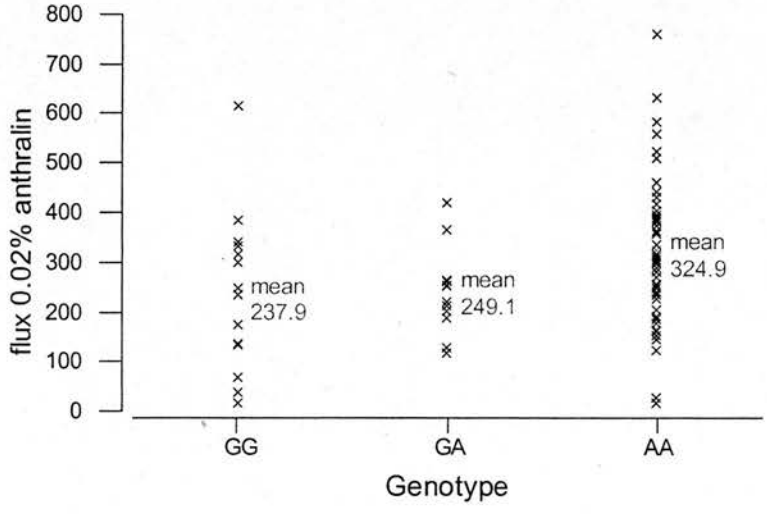


Figure 189. Flux induced by 0.02% Anthralin by ERCC1 exon 4 genotype.
Anthralin on lower back, flux measured at 48 hours

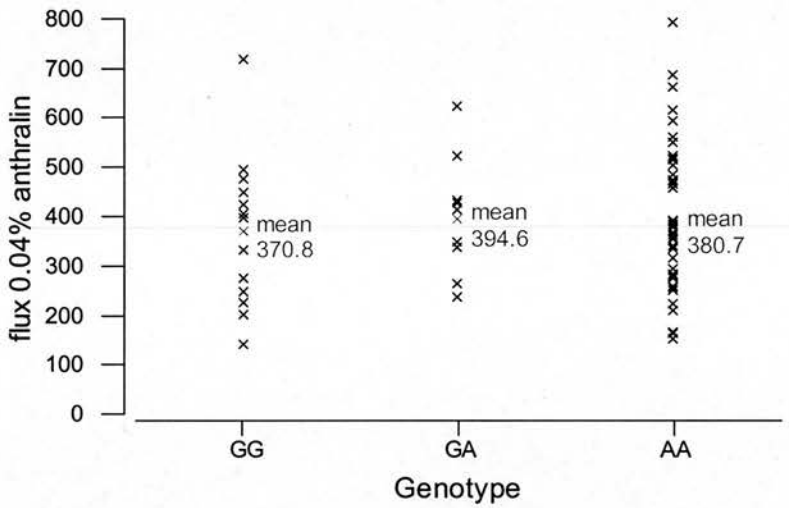


Figure 190 Flux induced by 0.04% Anthralin by ERCC1 exon 4 genotype.
Anthralin on lower back, flux measured at 48 hours

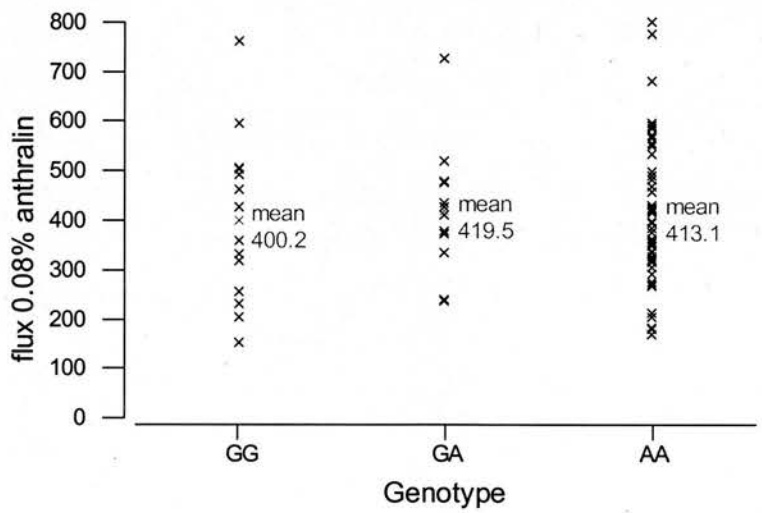


Figure 191 Flux induced by 0.08% Anthralin by ERCC1 exon 4 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and ERCC1 exon 4 genotype in group 1.

0.01% anthralin

Genotype	N	Mean	StDev	SE Mean
GG	14	111.4	89.0	23.8
GA	12	102.1	79.4	22.9
AA	50	189.7	121.8	17.2

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	117224	58612	4.76	0.011
Error	73	898856	12313		
Total	75	1016080			

0.02% anthralin

Genotype	N	Mean	StDev	SE Mean
GG	14	237.9	160.8	43.0
GA	12	249.1	93.2	26.9
AA	50	324.9	152.9	21.6

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	115495	57747	2.67	0.076
Error	73	1577335	21607		
Total	75	1692829			

0.04% anthralin

Genotype	N	Mean	StDev	SE Mean
GG	14	370.8	148.8	39.8
GA	12	394.6	112.1	32.4
AA	50	380.7	144.5	20.4

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	3681	1841	0.09	0.912
Error	73	1448514	19843		
Total	75	1452196			

0.08% anthralin

Genotype	N	Mean	StDev	SE Mean
GG	14	400.2	168.0	44.9
GA	12	419.5	130.8	37.8
AA	50	413.1	142.8	20.2

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2701	1350	0.06	0.939
Error	73	1553845	21286		
Total	75	1556546			

At the lowest dose of anthralin examined, 0.01%, there was significant variation between levels of flux and ERCC1 exon 4 genotypes. The AA genotype had significantly higher mean levels of flux response than GG and GA genotypes (p=0.01). However, analysis of factor and error values reveal that the majority of variation in response to anthralin is still due to factors other than genotype (SS(factor) 117224, compared with SS(error) of 898856, which contributes towards the majority of the SS(total) value of 1016080). As the strength of anthralin applied increases, so the level of significance in flux response by genotype decreases. At 0.04% and 0.08% there is almost no difference is observed in flux response between genotypes, with p values of 0.912 and 0.939 respectively. Here the variation which can be explained by genotype is even less than that seen at the lower doses. As the amount of DNA damage would be greater at higher anthralin doses, it would be expected that if the ERCC1 exon 4 polymorphism was associated with the flux response to anthralin, and thus the repair of such damage, such an association would reveal itself at the higher anthralin doses. As this was not the case, it seems that the apparent association of the polymorphism at the lowest dose (0.01%) was due to chance. No compelling evidence is seen here for the association of the ERCC1 exon 4

polymorphism with response to anthralin, and repair of DNA damage induced by anthralin.

XPF exon 11

Flux response to increasing doses anthralin by XPF exon 11 genotype in group 1.

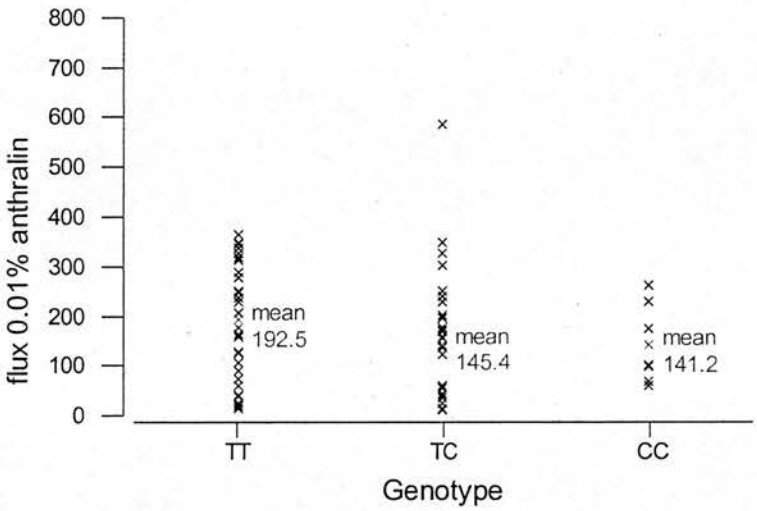


Figure 192. Flux induced by 0.01% Anthralin by XPF exon 11 genotype.

Anthralin on lower back, flux measured at 48 hours

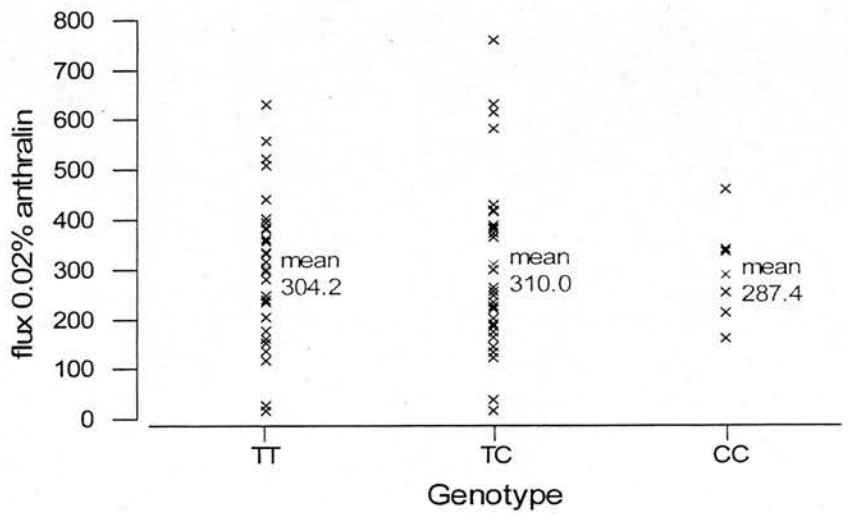


Figure 193 Flux induced by 0.02% Anthralin by XPF exon 11 genotype.

Anthralin on lower back, flux measured at 48 hours

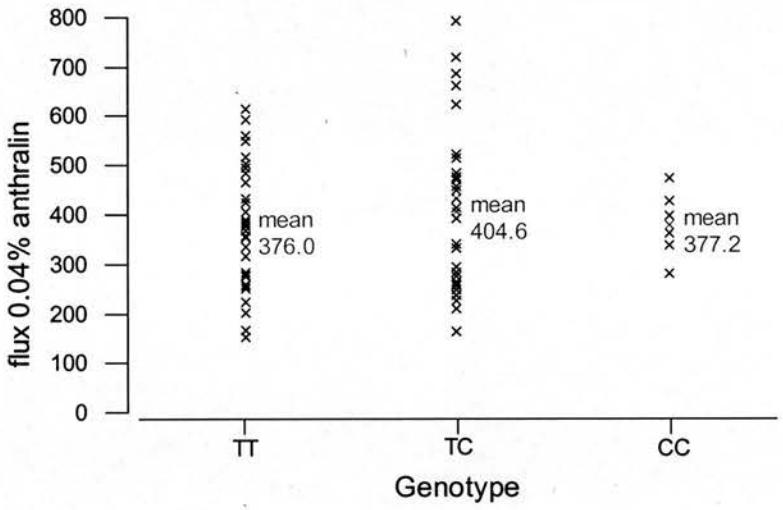


Figure 194 Flux induced by 0.04% Anthralin by XPF exon 11 genotype.
Anthralin on lower back, flux measured at 48 hours

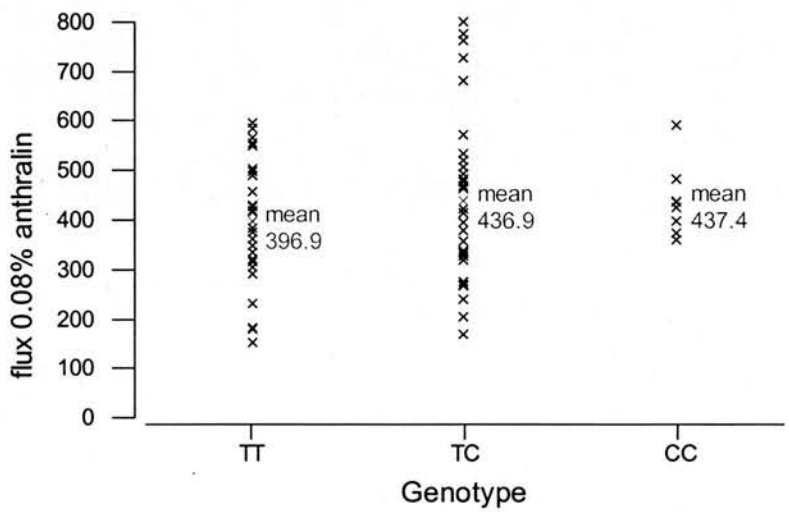


Figure 195. Flux induced by 0.08% Anthralin by XPF exon 11 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and XPF exon 11 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
TT	31	192.5	112.1	20.1
TC	32	145.4	127.5	22.5
CC	7	141.2	80.1	30.3

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	39741	19871	1.45	0.242
Error	67	919373	13722		
Total	69	959115			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
TT	31	304.2	144.3	25.9
TC	32	310.0	171.1	30.2
CC	7	287.4	99.3	37.5

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2996	1498	0.06	0.939
Error	67	1590660	23741		
Total	69	1593656			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
TT	31	376.0	125.6	22.6
TC	32	404.6	164.1	29.0
CC	7	377.2	62.7	23.7

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	14052	7026	0.35	0.704
Error	67	1331735	19877		
Total	69	1345787			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
TT	31	396.9	124.8	22.4
TC	32	436.9	172.0	30.4
CC	7	437.5	79.4	30.0

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	27716	13858	0.65	0.524
Error	67	1421846	21222		
Total	69	1449562			

The level of significance was not approached at any anthralin dose examined, p values ranged from 0.242 to 0.524. There was no pattern in the mean flux of a particular genotype at different genotypes. At 0.01% anthralin, the TT genotype group had the highest mean flux, while CC the lowest. The genotype with highest mean changes at each dose examined; at 0.08% TT has the lowest flux. The factor values for SS were much lower than the values for error at all doses, indicating that factors other than genotype are responsible for the variation seen in levels of flux. The XPF exon 11 polymorphism, from this data, is not associated with levels of flux following anthralin exposure.

Power calculations were performed using n=23 and n=7 for 0.01% and 0.08% anthralin. 0.01% had a between variance value of 19871, and a within variance values of 13722, which gave a power of 1 when n=23 and 0.97 when n==7. 0.08% had a between variance of 13858 and a within variance value of 21222. This gave a power of 0.99 when n=23 and 0.70 when n=7. The true power will lie between these values.

XPG exon 15

Flux response to increasing doses of anthralin by XPG exon 15 genotype in group 1.

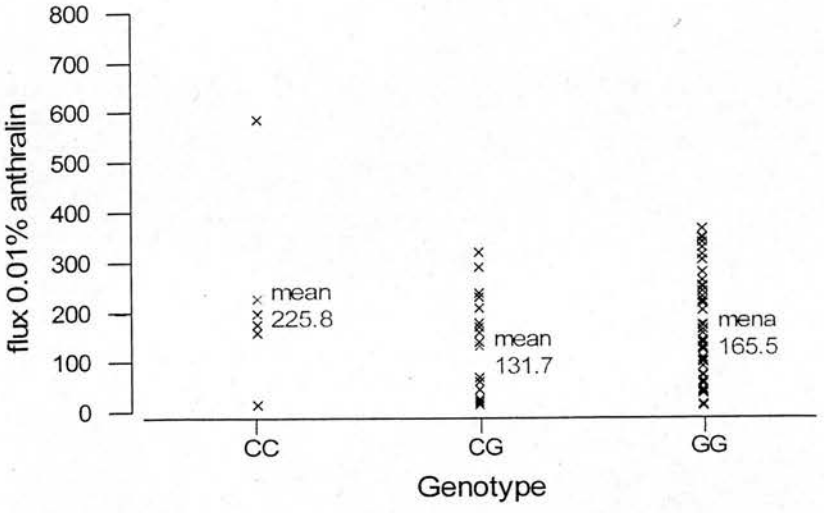


Figure 196. Flux induced by 0.01% Anthralin by XPG exon 15 genotype.

Anthralin on lower back, flux measured at 48 hours

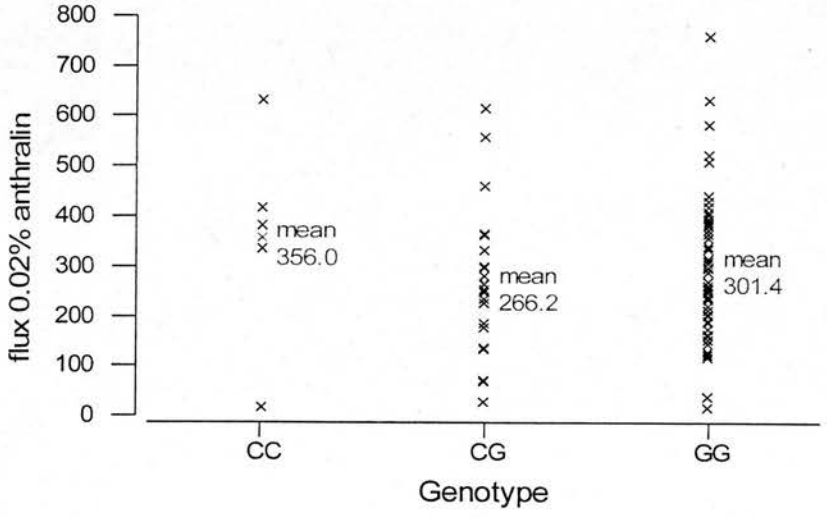


Figure 197. Flux induced by 0.02% Anthralin by XPG exon 15 genotype.

Anthralin on lower back, flux measured at 48 hours

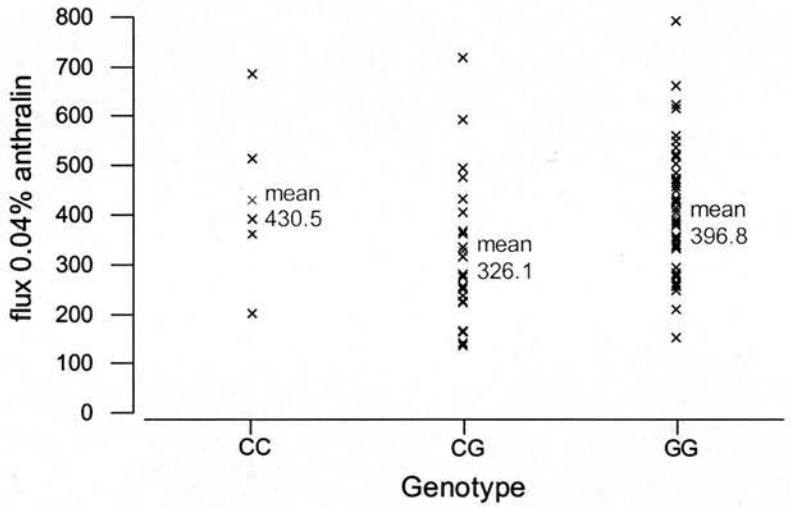


Figure 198 Flux induced by 0.04% Anthralin by XPG exon 15 genotype.
Anthralin on lower back, flux measured at 48 hours

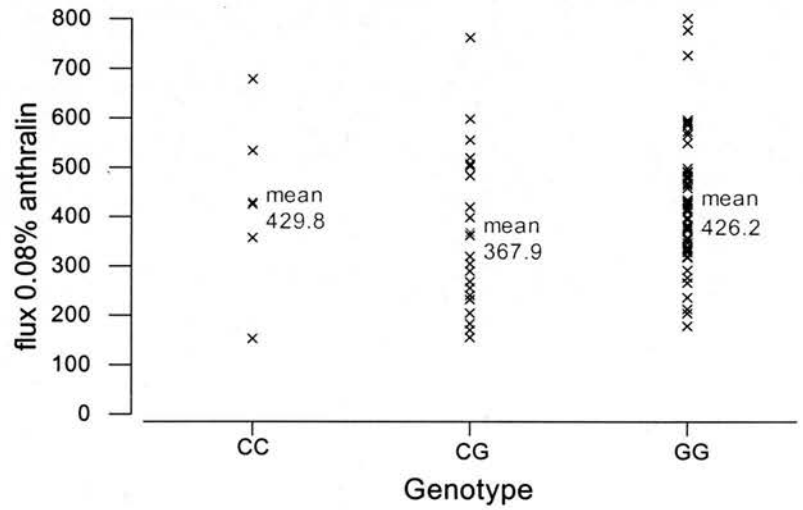


Figure 199 Flux induced by 0.08% Anthralin by XPG exon 15 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin by XPG exon 15 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	5	225.8	213.9	95.7
CG	21	131.7	98.2	21.4
GG	52	165.5	110.7	15.3

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	39993	19996	1.50	0.230
Error	75	1000809	13344		
Total	77	1040802			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	5	356.0	221.1	98.9
CG	21	266.2	151.4	33.0
GG	52	301.4	144.5	20.0

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	38177	19088	0.83	0.439
Error	75	1718390	22912		
Total	77	1756567			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	5	430.5	181.3	81.1
CG	21	326.1	152.2	33.2
GG	52	396.8	129.3	17.9

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	88360	44180	2.29	0.108

Error	75	1447825	19304
Total	77	1536185	

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	5	429.8	196.7	88.0
CG	21	367.9	163.6	35.7
GG	52	426.2	133.6	18.5

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	52732	26366	1.24	0.297
Error	75	1600785	21344		
Total	77	1653517			

Analysis of variance revealed no association between flux in response to anthralin and the XPG exon 15 genotype at any dose examined. P values ranged from 0.108 at 0.04% anthralin to 0.439 at 0.02%. Similarly, the factor values show little of the variation between flux responses can be attributed to the XPG genotype. This indicates that the XPG exon 15 polymorphism is not associated with flux response to, or repair of DNA damaged by, anthralin. XPG is a key component of the NER pathway, and has also been implicated in the repair of oxidative damage, which suggested it to be a good candidate for association with anthralin response, as anthralin induces oxidative stress. However, the exon 15 polymorphism does not appear to be associated with repair of oxidative damage, as indicated by the lack of association with flux following anthralin exposure.

Power calculations were carried out for 0.01% and 0.08% anthralin, using n=26 (balanced) and n=5 (most unbalanced). 0.01% had a between variance of 19996 and a within variance of 13344, which gave a power of 1 when n=26 and 0.87 when n=5. 0.08% had a between variance of 26366 and a within variance of 21344. Power was 1 when n=26, and 0.79 when n=5. The true power will lie between these values, but is sufficient to draw conclusions from the ANOVA tests.

XRCC1 exon 10

Flux response to increasing doses of anthralin by XRCC1 exon 10 genotype in group 1.

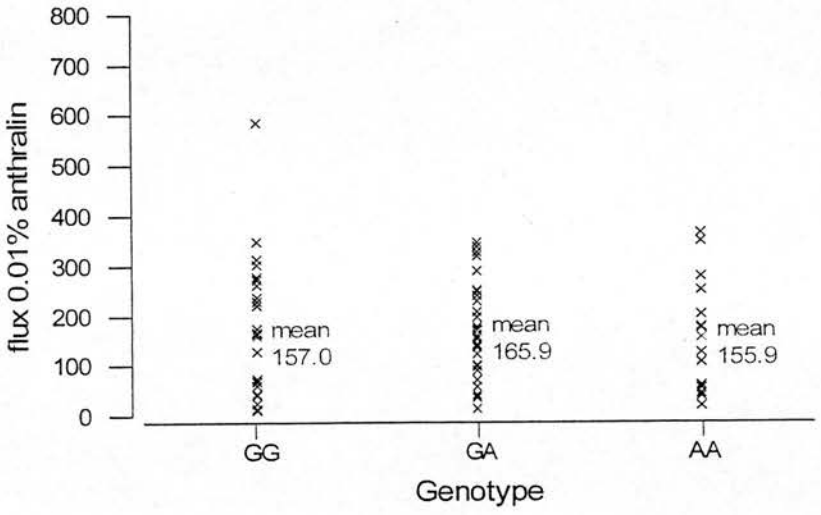


Figure 200 Flux induced by 0.01% Anthralin by XRCC1 exon 10 genotype.

Anthralin on lower back, flux measured at 48 hours

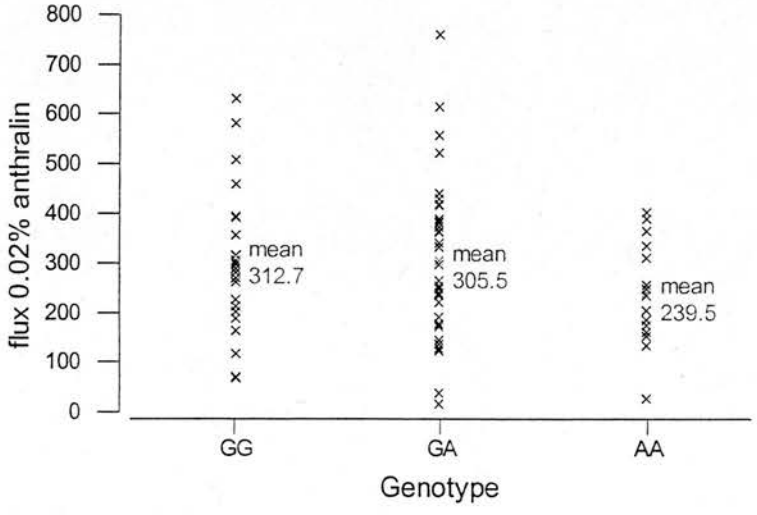


Figure 201. Flux induced by 0.02% Anthralin by XRCC1 exon 10 genotype.

Anthralin on lower back, flux measured at 48 hours

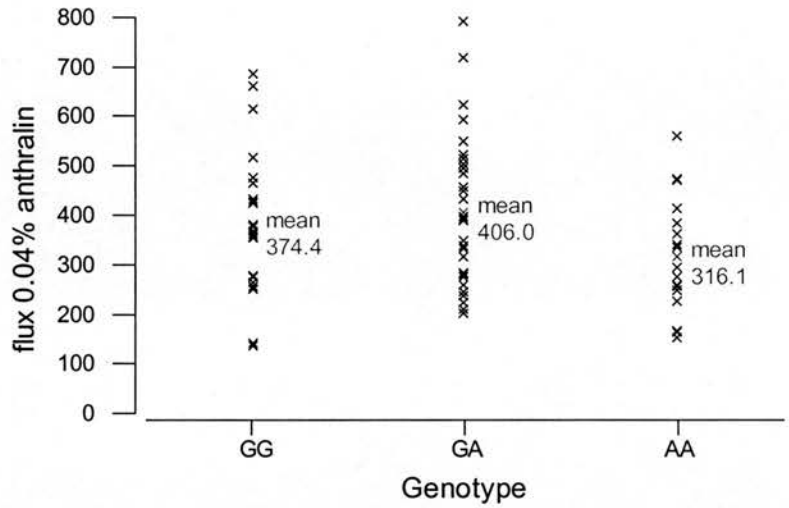


Figure 202 Flux induced by 0.4% Anthralin by XRCC1 exon 10 genotype.
Anthralin on lower back, flux measured at 48 hours

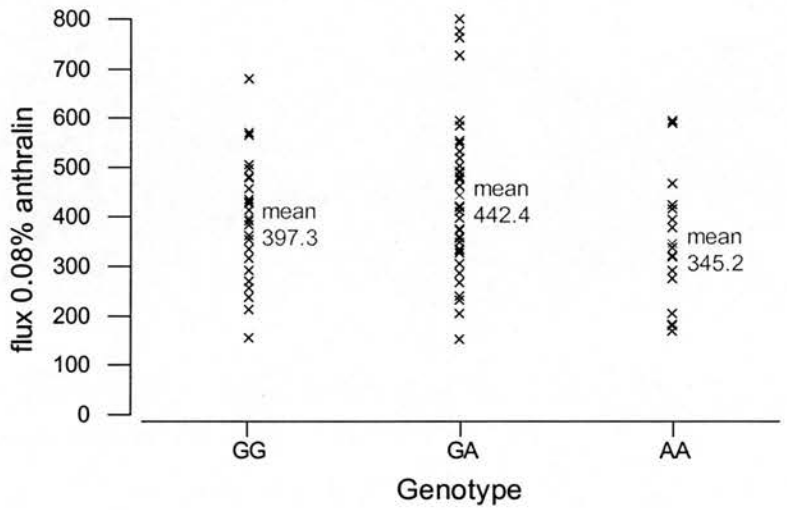


Figure 203. Flux induced by 0.08% Anthralin by XRCC1 exon 10 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and XRCC1 exon 10 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
GG	26	157.0	142.8	28.0
GA	36	166.0	100.3	16.7
AA	17	155.9	107.9	26.2

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1733	866	0.06	0.939
Error	76	1048628	13798		
Total	78	1050360			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
GG	26	312.7	152.5	29.9
GA	36	305.5	164.6	27.4
AA	17	239.5	104.3	25.3

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	64326	32163	1.43	0.245
Error	76	1703703	22417		
Total	78	1768028			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
GG	26	374.4	141.2	27.7
GA	36	406.0	143.1	23.9
AA	17	316.1	117.3	28.5

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	93409	46705	2.47	0.091
Error	76	1435952	18894		
Total	78	1529361			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
GG	26	397.3	120.6	23.6
GA	36	442.4	159.7	26.6
AA	17	345.2	129.4	31.4

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	112585	56292	2.81	0.067
Error	76	1524289	20056		
Total	78	1636874			

The XRCC1 exon 10 polymorphism showed no formal association with flux response at any dose of anthralin examined. The AA homozygous genotype had lower mean values of flux at all doses, whilst the heterozygous GA genotype had the highest mean at all doses except at 0.02%. At the higher doses of 0.04% and 0.08% anthralin, the genotype variation of response approached the formal level of significance, p=0.05. At 0.04% anthralin, the p value was 0.091, and at 0.08%, p=0.067. Comparison of factor and error reveals most variation of flux response is still due to factors other than genotype.

Power calculations were carried out for 0.01% and 0.08% anthralin, using n=26 (balanced) and n=17 (most unbalanced). Between variance was 866 for 0.01% and within variance was 13798. Power was 0.33 when n=26 and 0.23 when n=17. 0.08% gave a between variance value of 56292 and a within variance value of 20056. Power was 1 both when n=26 and when n=17. The power at the lower anthralin dose was extremely low, although it was good at 0.08%. An increase in numbers of individuals studied would benefit the power of the ANOVA tests for lower anthralin doses.

XRCC1 is required to ligate the newly synthesised DNA to the damaged strand on completion of the BER pathway. As BER repair oxidative damage, this polymorphism more than any other was thought to be most likely to show an association with flux response. The lack of formal significance means no conclusive association can be drawn between the XRCC1 genotype and the repair of anthralin-induced damage, as indicated by flux response. It would be of interest to repeat this study in a larger group, perhaps including higher doses of anthralin to see if significance increased. However, higher doses could result in confounding skin and DNA damage, which could stop the flux from being accurately measured.

XRCC3 exon 7

Flux response to increasing doses anthralin by XRCC3 exon 7 genotype in group 1.

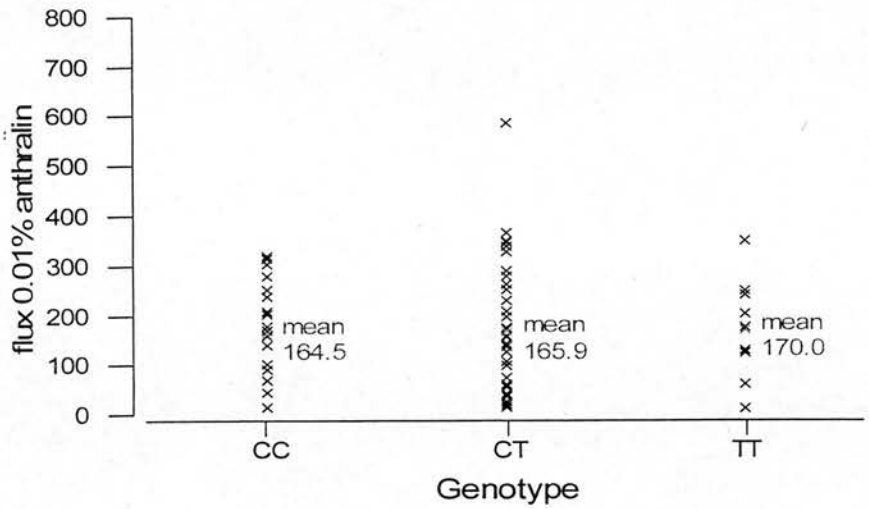


Figure 204. Flux induced by 0.01% Anthralin by XRCC3 exon 7 genotype.
Anthralin on lower back, flux measured at 48 hours

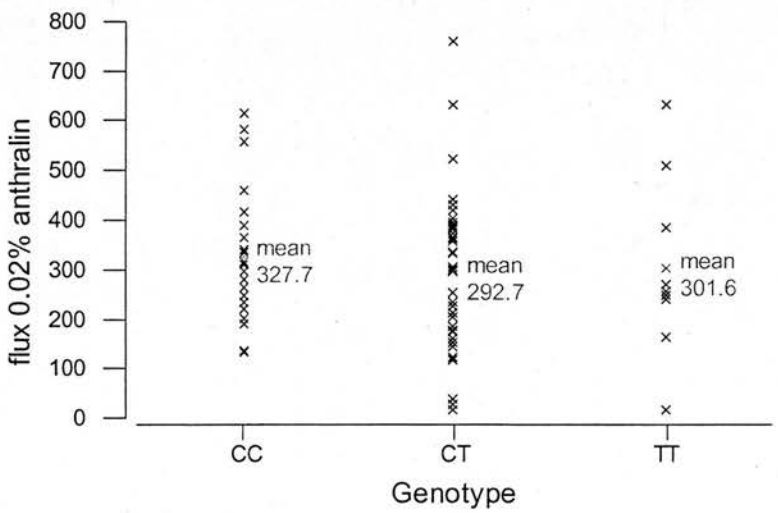


Figure 205. Flux induced by 0.02% Anthralin by XRCC3 exon 7 genotype.
Anthralin on lower back, flux measured at 48 hours

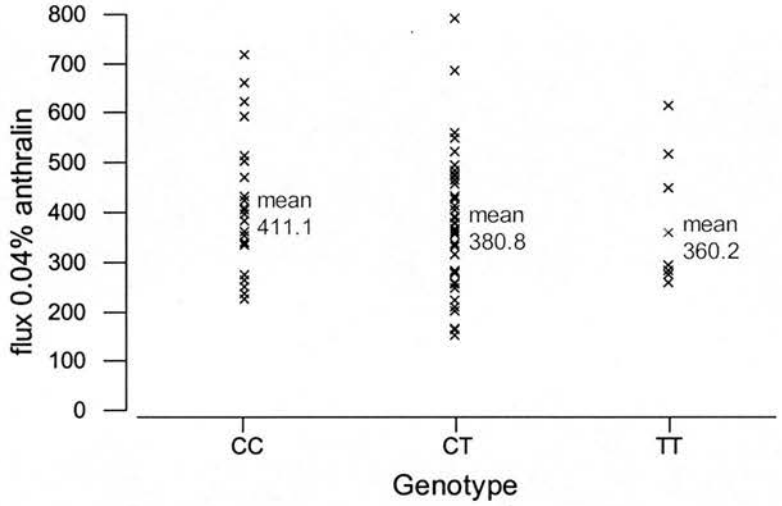


Figure 206 Flux induced by 0.04% Anthralin by XRCC3 exon 7 genotype.

Anthralin on lower back, flux measured at 48 hours

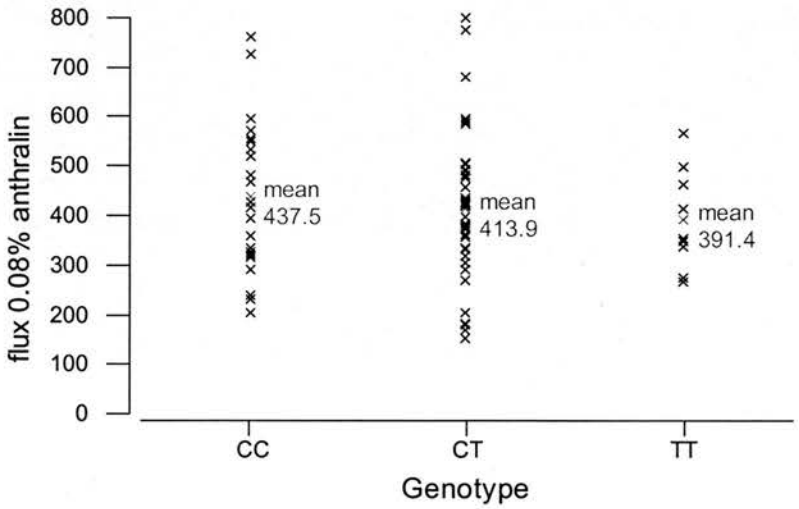


Figure 207 Flux induced by 0.08% Anthralin by XRCC3 exon 7 genotype.

Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and XRCC3 exon 7 genotype in group 1.

0.01% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	22	164.5	95.9	20.4
CT	40	165.9	132.1	20.9
TT	9	170.0	103.1	34.4

Analysis of variance of flux from 0.01% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	193	96	0.01	0.993
Error	68	959008	14103		
Total	70	959201			

0.02% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	22	327.7	133.8	28.5
CT	40	292.7	154.7	24.5
TT	9	301.6	183.2	61.1

Analysis of variance of flux from 0.02% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	17490	8745	0.38	0.687
Error	68	1577493	23198		
Total	70	1594983			

0.04% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	22	411.1	142.4	30.4
CT	40	380.8	140.6	22.2
TT	9	360.2	132.6	44.2

Analysis of variance of flux from 0.04% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	20761	10381	0.53	0.592
Error	68	1337848	19674		
Total	70	1358609			

0.08% Anthralin

Genotype	N	Mean	StDev	SE Mean
CC	22	437.5	153.6	32.7
CT	40	413.9	148.5	23.5
TT	9	391.1	101.5	33.8

Analysis of variance of flux from 0.08% anthralin between genotypes:

Source	DF	SS	MS	F	P
Factor	2	15558	7779	0.37	0.694
Error	68	1438267	21151		
Total	70	1453824			

At none of the doses of anthralin examined was there an association of the XRCC3 exon 7 genotype and flux response (p values 0.993 to 0.592). On comparison of factor and error, the genotype explained very little of the total variation of flux, for example, at 0.01% anthralin the SS(factor) value was 193, SS(error) 1438267, and SS(total) 959201. At 0.08% anthralin, the SS(factor) 1555, SS(error) 1438267, making a SS(total) value of 1453824. From this data, there is no evidence that the XRCC3 exon 7 polymorphism is associated with flux response to anthralin, indicating that this polymorphism is not involved with the response to oxidative stress.

Power calculations were carried out for 0.01% and 0.08% anthralin, using n=24 (balanced) and n=9 (least balanced). 0.01% had a between variance value of 96 and a within variance value of 14103. This gave a power of .017 when n=24 and 0.06 when n=9. 0.08% had a between variance value of 7779 and a within variance of 21151. Power was 0.97 when n=24 and .057 when n=9. The lower dose has poor power, which could be increased by a larger study group.

The GSTT1 deletion leads to a null allele, which produces no functional protein. In order to genotype samples for this polymorphism, the PCR assay described by Kerb *et al* (2002) was initially attempted. However, it was not possible to achieve PCR products with these primers, either under the published conditions, or when these conditions were modified. From the NCBI sequence, new primer pairs were designed. The null primers were situated either side of the deletion region. In the wild-type allele, these primers are too far apart (approximately 50kb) for a PCR product to be produced under these conditions. However, in the null allele much of the intervening sequence is deleted, and a product of 1498 bp was produced. The wild-type primers were situated within the deleted region. The null allele did not produce a PCR product with these primers, while in the wild-type allele, a product of 466bp was amplified.

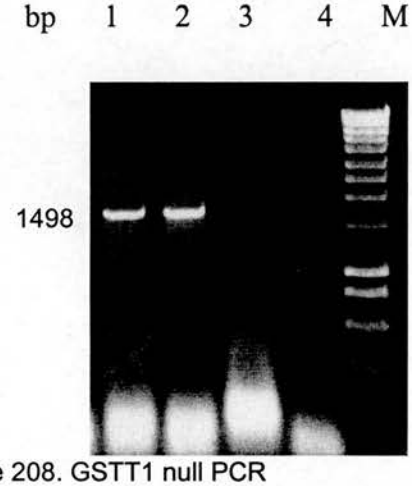


Figure 208. GSTT1 null PCR
Lane1, 2 PCR product formed, null allele present
Lane 3 no product, no null allele

Lane 4, negative control

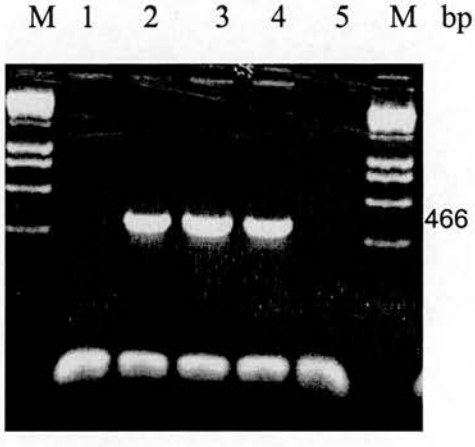


Figure 209 GSTT1 wild type PCR
Lane 1 no PCR product, no w/t allele
Lanes 2-4 PCR product, w/t allele present
Lane 5, negative control

GSTT1 and erythemal response

Erythema induced by incremental doses of UVR by GSTT1 genotype in group 1.

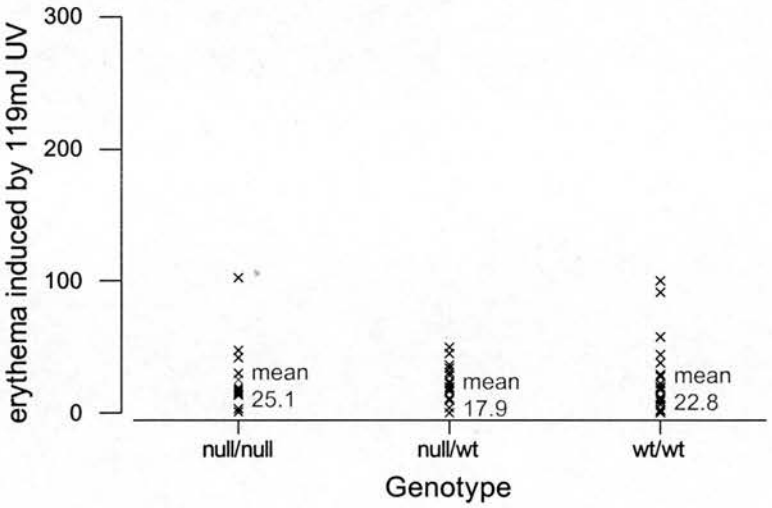


Figure 210. Erythema induced by 119mJ per cm^2 UV by GSTT1 genotype.
UV on lower back, measured at 48 hours

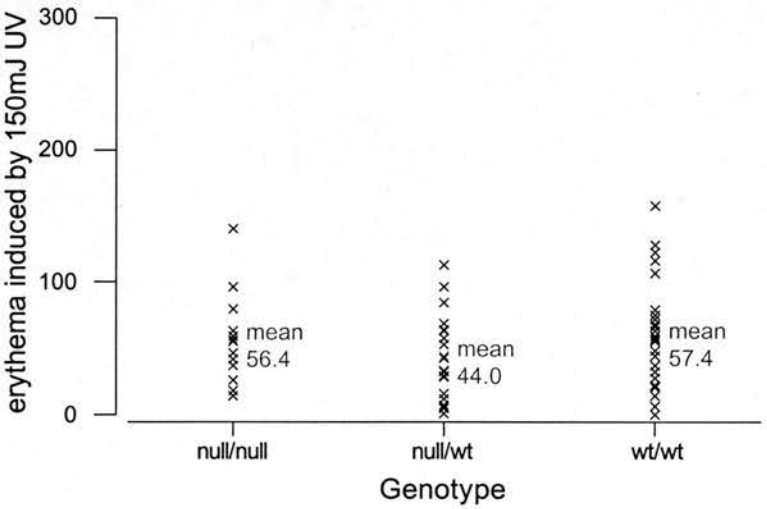


Figure 211 Erythema induced by 150mJ per cm^2 UV by GSTT1 genotype.
UV on lower back, measured at 48 hours

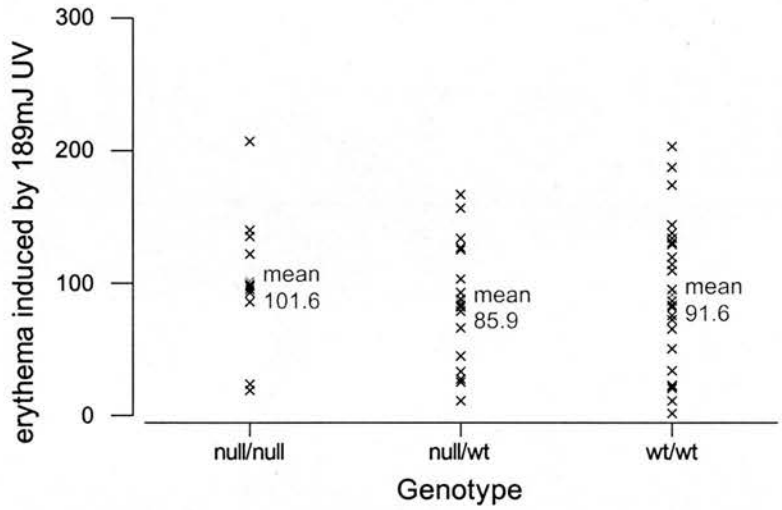


Figure 212. Erythema induced by 189mJ per cm^2 UV by GSTT1 genotype.
UV on lower back, measured at 48 hours

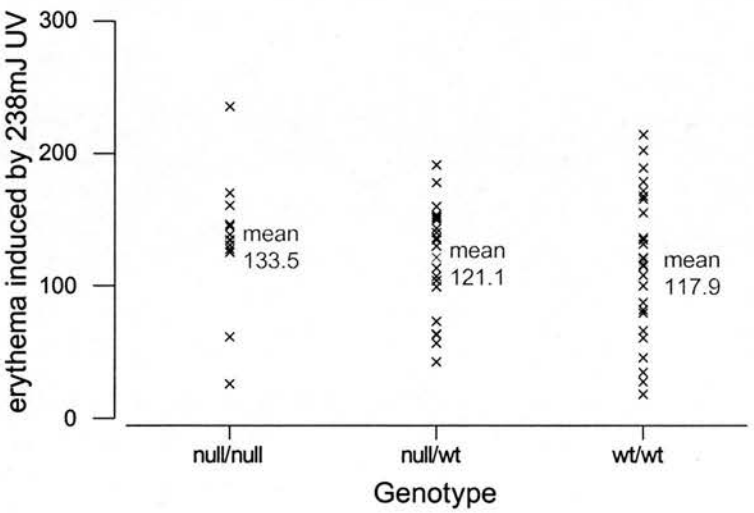


Figure 213 Erythema induced by 238mJ per cm^2 UV by GSTT1 genotype.
UV on lower back, measured at 48 hours

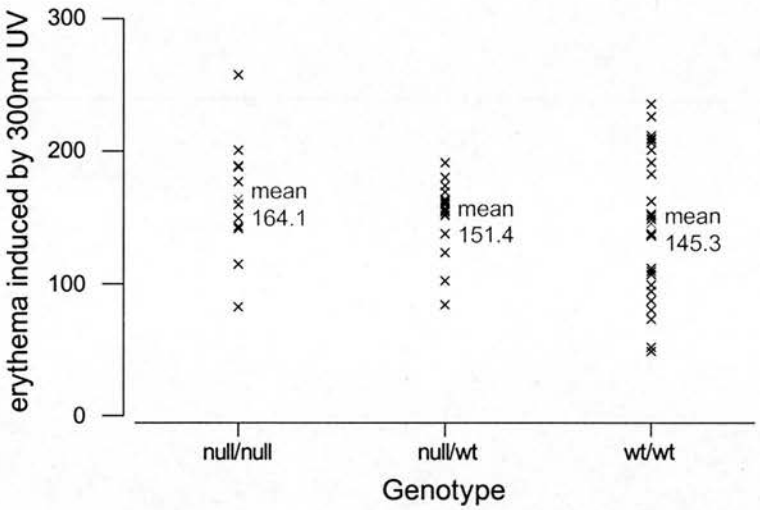


Figure 214. Erythema induced by 300mJ per cm^2 UV by GSTT1 genotype.

UV on lower back, measured at 48 hours

Analysis ofGSTT1 genotype and erythemal response to UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
null/null	12	25.14	28.60	8.26
null/wt	21	17.95	15.33	3.35
wt/wt	27	22.79	25.39	4.89

Analysis of variance of levels of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	469	234	0.44	0.647
Error	57	30463	534		
Total	59	30932			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
null/null	12	56.4	35.8	10.3
null/wt	21	44.04	31.51	6.88
wt/wt	27	57.45	40.79	7.85

Analysis of variance of levels of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2345	1172	0.87	0.426
Error	57	77216	1355		
Total	59	79560			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
null/null	12	101.6	49.9	14.4
null/wt	21	85.90	42.16	9.20
wt/wt	27	91.6	54.0	10.4

Analysis of variance of levels of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1877	939	0.39	0.682
Error	57	138645	2432		
Total	59	140522			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
null/null	12	133.5	51.9	15.0
null/wt	21	121.08	41.83	9.13
wt/wt	27	117.9	54.3	10.5

Analysis of variance of levels of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2061	1030	0.42	0.662
Error	57	141399	2481		
Total	59	143460			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
null/null	12	164.1	44.3	12.8
null/wt	21	151.37	24.65	5.38
wt/wt	27	145.3	54.7	10.5

Analysis of variance of levels of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2918	1459	0.75	0.479
Error	57	111471	1956		
Total	59	114388			

At none of the UV doses examined was there a significant difference in erythemal response between genotypes. At the higher UV doses, of 189, 238 and 300mJ per cm^2, individuals in the group homozygous for the null allele did display higher mean levels of

UV-induced erythema, while those homozygous for the wild-type allele had the lowest mean erythemal response at 238 and 300mJ per cm^2. This was, however, not significant at any dose, with p values ranging form 0.682 to 0.426. Analysis of the factor and error values reveal how little of the total amount of variation can be attributed to the presence or absence of the GSTT1 null allele, for example, at 238mJ per cm^2, the SS(factor) value is 2061, while the SS(error) value, which comprises all the variation due to factors other than genotype, was 141399, contributing most of the SS(total) value of 143460. At 300mJ per cm^2 there is a similar picture, with the SS(factor), the variation which can be explained by genotype , the value being 2918, while the SS(error) is 111471, and the SS(total) being 114388.

Power calculations were performed for group 1 using n=20 (balanced) and n=12 least balanced). 119mJ UV had a between variance value of 234 and a within variance value of 534. When n=20, the power was 0.96, and 0.80 when n=12. The between variance value at 300mJ was 1459 while the within variance value of 1956, giving power of 0.99 when n=290 and 0.96 when n=12. The true power of the ANOVA calculations will lie between these values, but is high enough to accept the p values.

The study by Kerb *et al* (2002) would suggest that individuals with the null/null genotype would be expected to display significantly higher erythemal responses to UVR than those with only one deleted GSTT1 allele and those homozygous for the functional wild-type allele. Their finding, of those homozygous for the null allele having lower MEDs, and this being more sensitive to UV, than the heterozygotes and wild-type homozygotes was not reinforced by this study which used a more analytical measure of sensitivity to UV. Kerb and colleagues studied 110 German Caucasians, being a larger study than this. Chi-square analysis of the genotype frequencies reported by Kerb *et al* compared with those obtained in this study revealed a significant difference between the two groups (DF=2, p=0.033). Because of this difference in the genotype frequency, it is difficult to confirm or deny the findings of Kerb *et al*. The MED is not an accurate method of determining UV sensitivity when compared with the use of a reflectance meter, human judgment errors can be introduced into the equation when determining MED, compared with the numerical outcome of the reflectance meter. Due to the limitations of the MED, it would be expected that the findings of this study, which see no association between the GSTT1

polymorphism and UV sensitivity is likely to give a more accurate picture as to the role of the GSTT1 polymorphism in determining UV sensitivity. What can be concluded, however, is that there is no association between the GSTT1 polymorphism and UV sensitivity in this study.

GSTT1 and response to anthralin

Flux response to increasing doses anthralin by GSTT1 genotype in group 1.

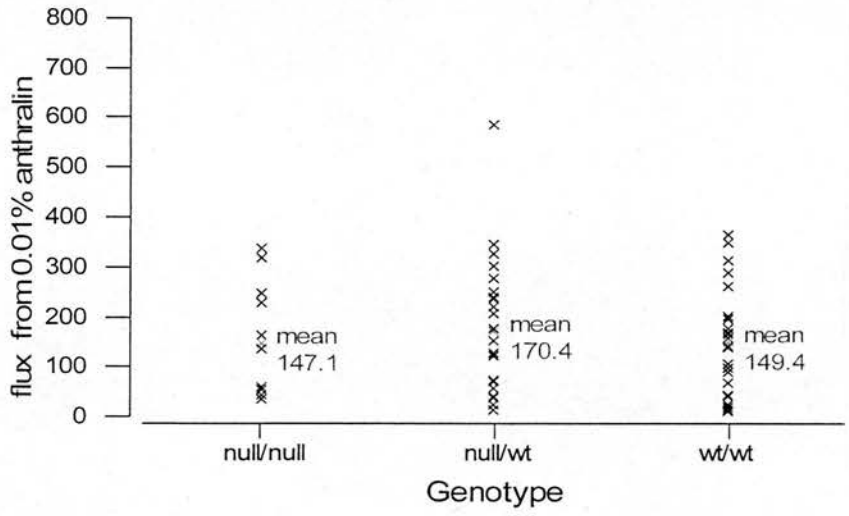


Figure 215. Flux induced by 0.01% Anthralin by GSTT1 genotype.
Anthralin on lower back, flux measured at 48 hours

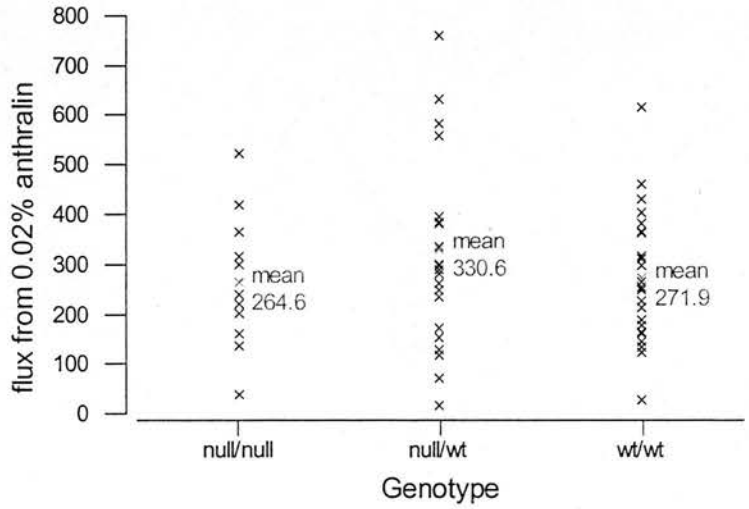


Figure 216 Flux induced by 0.02% Anthralin by GSTT1 genotype.
Anthralin on lower back, flux measured at 48 hours

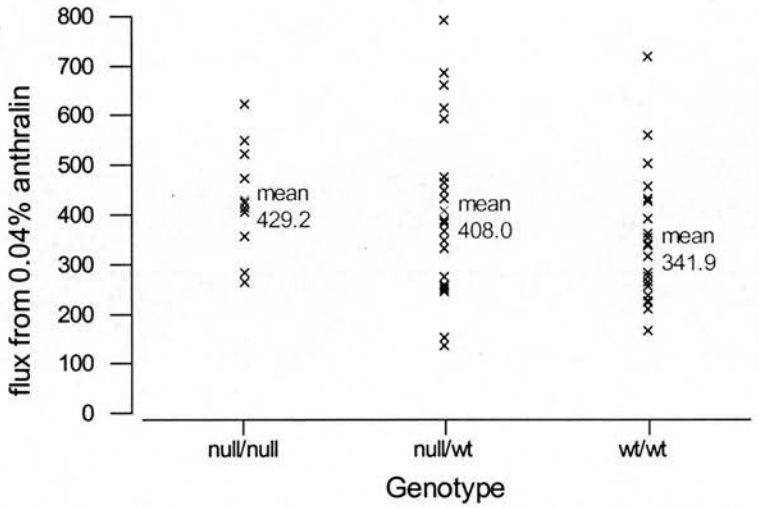


Figure 217. Flux induced by 0.04% Anthralin by GSTT1 genotype.
Anthralin on lower back, flux measured at 48 hours

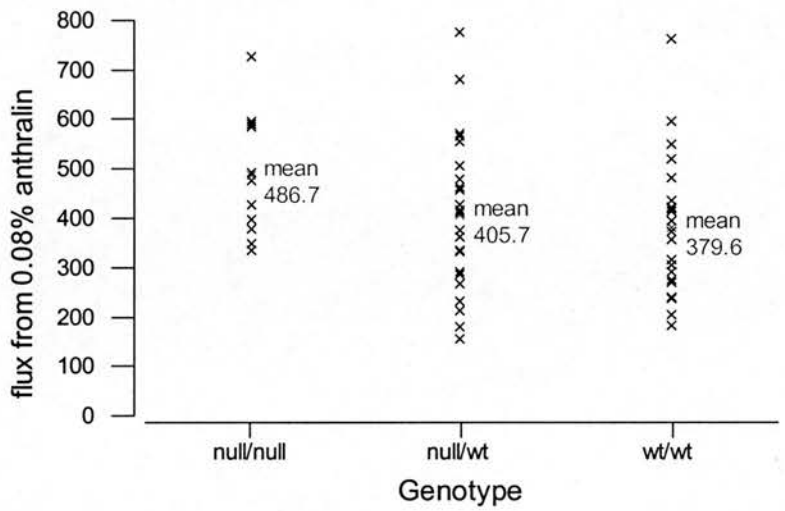


Figure 218 Flux induced by 0.08% Anthralin by GSTT1 genotype.
Anthralin on lower back, flux measured at 48 hours

Analysis of flux response to anthralin and GSTT1 genotype in group 1.

0.01% anthralin

Genotype	N	Mean	StDev	SE Mean
null/null	11	147.1	120.2	36.3
null/wt	23	170.4	141.5	29.5
wt/wt	23	149.4	108.3	22.6

Analysis of variance of flux induced by 0.01% anthralin between GSTT1 genotypes:

Source	DF	SS	MS	F	P
Factor	2	6497	3248	0.21	0.813
Error	54	842942	15610		
Total	56	849439			

0.02% anthralin

Genotype	N	Mean	StDev	SE Mean
null/null	11	264.6	137.6	41.5
null/wt	23	330.6	193.9	40.4
wt/wt	23	271.9	133.8	27.9

Analysis of variance of flux induced by 0.02% anthralin between GSTT1 genotypes:

Source	DF	SS	MS	F	P
Factor	2	51450	25725	0.98	0.380
Error	54	1410513	26121		
Total	56	1461962			

0.04% anthralin

Genotype	N	Mean	StDev	SE Mean
null/null	11	429.2	108.4	32.7
null/wt	23	408.0	171.1	35.7
wt/wt	23	341.9	129.5	27.0

Analysis of variance of flux induced by 0.04% anthralin between GSTT1 genotypes:

Source	DF	SS	MS	F	P
Factor	2	76426	38213	1.83	0.171
Error	54	1130513	20935		
Total	56	1206939			

0.08% anthralin

Genotype	N	Mean	StDev	SE Mean
null/null	11	486.7	124.8	37.6
null/wt	23	405.7	158.5	33.0
wt/wt	23	379.6	143.5	29.9

Analysis of variance of flux induced by 0.08% anthralin between GSTT1 genotypes:

Source	DF	SS	MS	F	P
Factor	2	86430	43215	2.01	0.144
Error	54	1161038	21501		
Total	56	1247468			

No association was observed between the GSTT1 polymorphism and flux response following exposure to anthralin at any dose examined. P values ranged from 0.813 at the lowest dose of 0.01% to 0.144 at the highest dose of 0.08%. Analysis of factor and error values reveal that the majority of error is due to other factors than genotype, implying that the GSTT1 protein is not associated with anthralin response, or in the detoxification of cells following exposure to anthralin.

Power calculations were performed at 0.01% anthralin and 0.08% anthralin, using n=19 (balanced) and n=11 (most unbalanced). The between variance value at 0.01% was 3248 and within variance was 15610. Power was 0.68 when n=19 and 0.43 when n=11.The between variance value at 0.08% anthralin was 43215, and within variance 21501. Power when n=19 was 1, and 0.99 when n=11. The power at the higher anthralin dose is good, but rather low at 0.01%, In future studies, one would want to increase the power in order to have more confidence in ANOVA tests, which could be achieved by increasing sample size.

Genotypes were determined using a RFLP assay, which utilised the BstUI restriction site, which spans the codon 72 polymorphism. This was based on the assay first described by Ara *et al* (1990) using *Acc*II, an isochizomer of *Bst*UI. Using the primers and conditions detailed in Chapter 2 (methods) a PCR reaction was carried out, giving a PCR product of 296 base pairs. The Arg allele contains the *Bst*UI restriction site, CG/CG, and under appropriate digestion conditions yields two fragments of 169 and 127 bp. After digestion, fragments were separated by electrophoresis through a 2% low melting point agarose gel stained with ethidium bromide, and visualised with a UV source. The Pro allele does not contain the restriction site, and gives a single band of 296 bp, while the heterozygous genotype (Arg/Pro) gave three bands of 296, 169 and 127 bp.

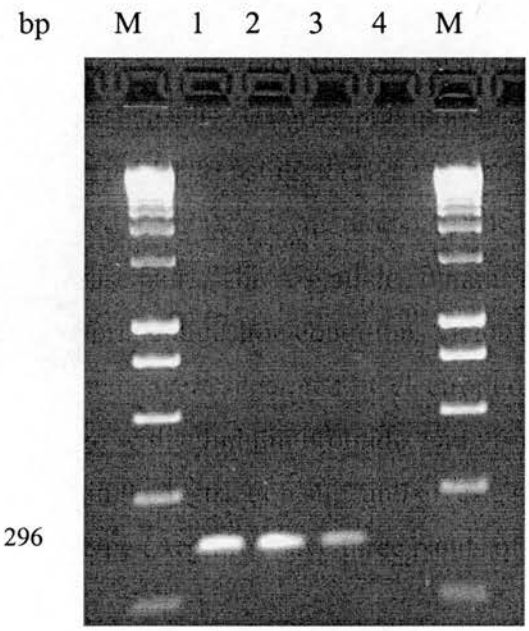


Figure 219, p53 codon 72 PCR products

Lanes 1-3, PCR products
Lane 4, negative control

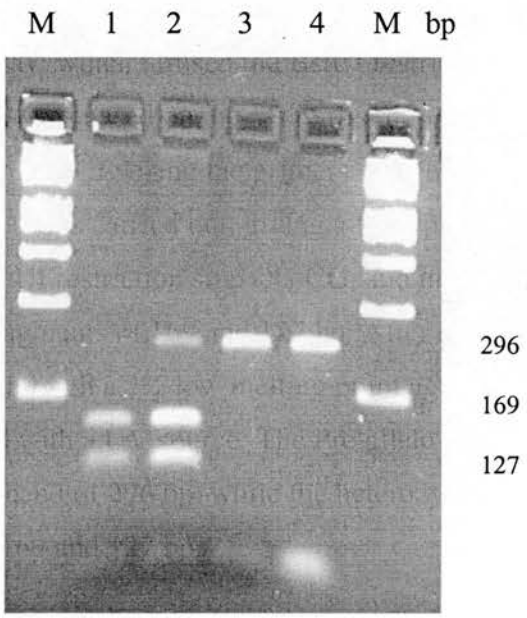


Figure 220, p53 codon 72 digest

Lane 1 Arg/Arg
Lane 2 Arg/Pro
Lane 3 Pro/Pro
Lane 4 undigested PCR product

p53 codon 72 Genotype frequencies

Genotype	Group 1(%)	Group 2(%)
Arg/Arg	37/74 (50.00)	13/31 (41.94)
Arg/Pro	31/74 (41.89)	16/31 (51.61)
Pro/Pro	6/74 (8.11)	2/31 (6.45)
Total	74 (100)	31 (100)

No statistically significant difference was observed in genotype frequencies between the two study groups (χ^2 DF = 2, P-Value = 0.658).

Allele frequency at codon 72 has previously been shown to correlate with latitude (Beckman *et al*, 1994). It was proposed that the codon 72 polymorphism is balanced and maintained by natural selection, and that alleles encoding a proline at codon 72 might be selected for in environments subject to high levels of UV. Their rationale was that they saw a marked ethnic differentiation, a strong correlation with latitude, and the similarity of allele frequencies between Indians (from Sri Lanka, Tamil Nada and Keralda) and African Blacks (Nigerians), who, they claim, show little genetic similarity apart from dark skin pigmentation. If the p53 codon 72 polymorphism is indeed maintained by natural selection, and the proline allele selected for in environments where the population are exposed to high levels of UVR, the proline allele would have to confer some level of evolutionary advantage to this population, such as giving an increased tolerance to UVR and a level of protection from its deleterious effects. The two study groups investigated here are fairly homogenous in ethnic mix and are typical of an Edinburgh population. The latitude of Edinburgh provides little exposure to high levels of UVR, and if the Pro allele did confer some advantage in populations exposed to high levels of UVR, there would be no reason for this allele to be selected for in this environment, as it would confer little of no evolutionary advantage where there is little or no exposure to UVR. The frequency of the proline allele would therefore be expected to the lower in a Scottish study group compared to in an African study group, as there would be no reason for it to be selected for, with a corresponding increase in the frequency of the arginine allele in the Scottish group.

As described in the table above, the Arginine allele is the predominant allele found in both study groups, with individuals homozygous for the Proline allele being observed on only 6 occasions out of 74 individuals studied in group 1 (8.11%) and 2 occasions out of 31 in group 2 (6.45%). That these allele frequencies agree with the observed correlation between higher latitude and increased frequency of the Arg allele does not however automatically imply that the Pro allele might offer protection against high levels of UVR. It is likely that there is an independent, and confounding, association with skin colour, which may or may not be involved in protection against UVR. This view is supported by work from Bastiaens and colleagues (2001). UVR is a known risk factor for development of non-melanoma skin cancers, therefore, if the pro allele did offer a level of protection against UVR, it would be expected that there would be a statistically significant difference in genotype frequency in individuals with a variety of cutaneous malignancies, and those without. Bastiaens *et al* studied individuals from the Netherlands with a variety of cutaneous malignancies. 86 subjects with squamous cell carcinoma, 168 healthy controls, 121 subjects with basal cell carcinoma and 108 with non-familial malignant melanoma were genotyped for the p53 codon 72 polymorphism. Genotype frequencies were similar in each group, with the breakdown of the SCC group being 47.1% homozygous for the arginine allele, 46.0% heterozygous for the two alleles, and 6.9% homozygous for the proline allele, versus 47.8%, 45.8% and 6.4% respectively in the control group. No statistically significant difference in genotype frequencies was seen between the SCC group and the controls, or between the BCC and non-familiar malignant melanoma groups and controls, suggesting that harbouring homozygosity for the arginine allele does not appear to represent a significant risk factor for SCC. That this does not agree with the view of both McGregor *et al*, and Shen *et al*, highlights the necessary for further investigation of this polymorphism and sensitivity to UVR.

In each of two independent study groups, individuals were exposed to a range of UV doses, on either the lower back in group 1, or the inner forearm in group 2. UVB-induced erythema was plotted against genotype (Arg/Arg, Arg/Pro, or Pro/Pro) and analysed for variance in levels of erythema between genotypes for each dose.

Erythema induced by incremental doses of UVR by p53 codon 72 genotype in group 1.

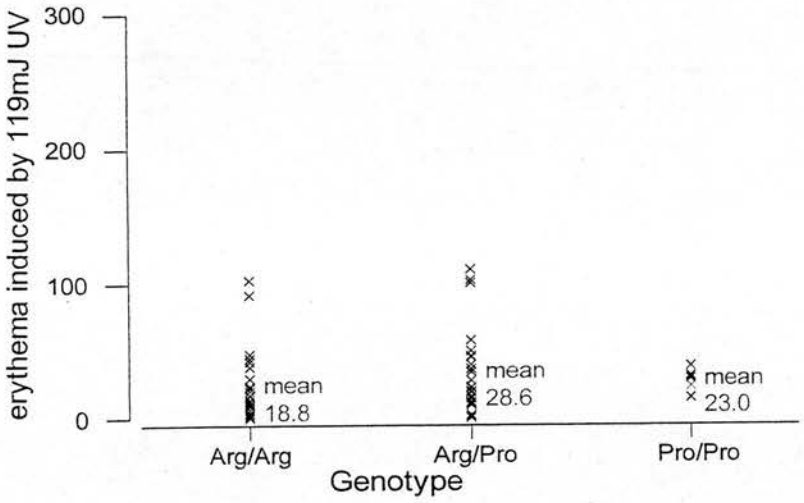


Figure 221 Erythema induced by 119mJ per cm^2 UV by p53 codon 72 genotype.

UV on lower back, measured at 48 hours

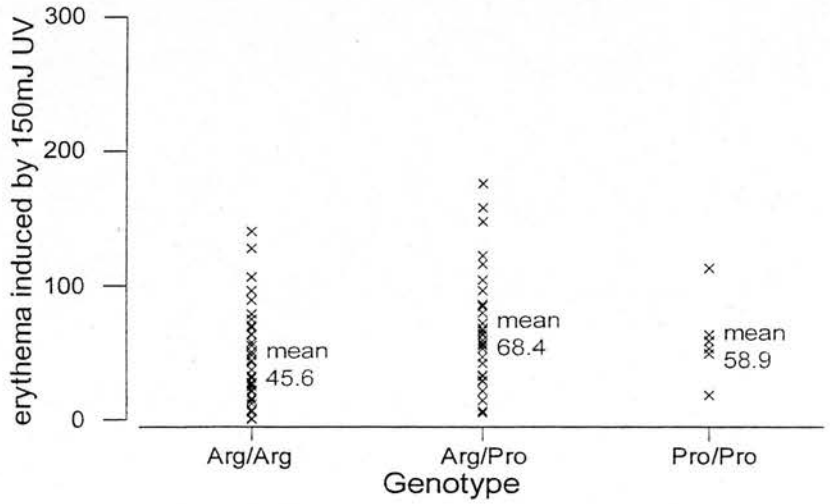


Figure 222 Erythema induced by 150mJ per cm^2 UV by p53 codon 72 genotype.

UV on lower back, measured at 48 hours

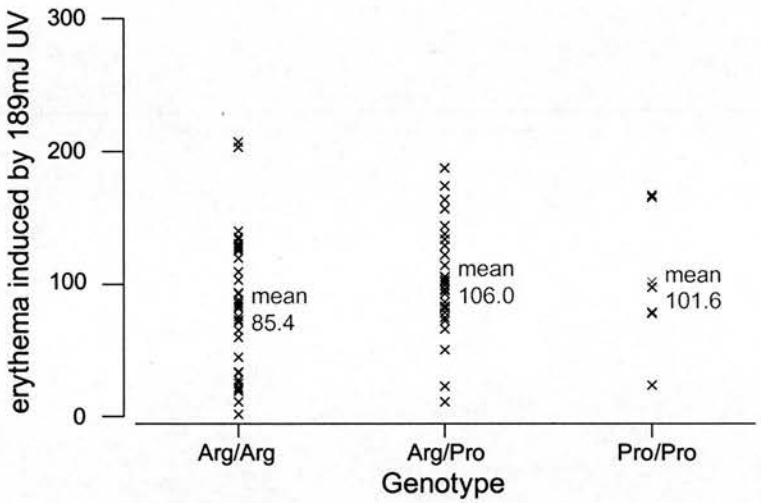


Figure 223 Erythema induced by 189mJ per cm^2 UV by p53 codon 72 genotype.
UV on lower back, measured at 48 hours

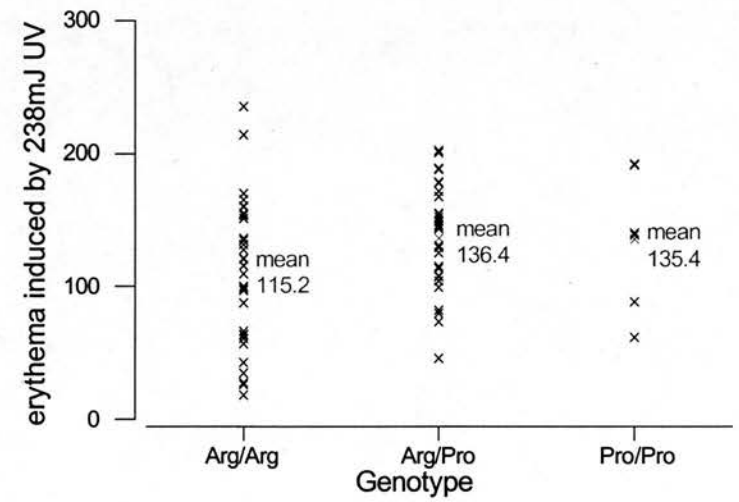


Figure 224 Erythema induced by 238mJ per cm^2 UV by p53 codon 72 genotype.
UV on lower back, measured at 48 hours

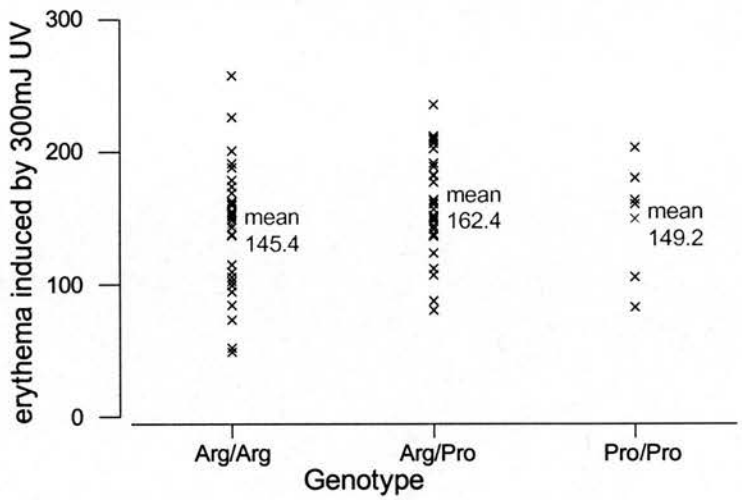


Figure 225 Erythema induced by 300mJ per cm^2 UV by p53 codon 72 genotype.
UV on lower back, measured at 48 hours

Analysis of p53 codon 72 genotype and erythemal response UVR for each dose in group 1.

119mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	37	18.81	22.91	3.77
Arg/Pro	31	28.62	29.58	5.31
Pro/Pro	6	22.97	14.88	6.07

Analysis of variance of erythema induced by 119mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	1627	813	1.25	0.293
Error	71	46234	651		
Total	73	47861			

150mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	37	45.61	35.12	5.77
Arg/Pro	31	68.44	42.60	7.65
Pro/Pro	6	58.9	30.7	12.5

Analysis of Variance of erythema induced by 150mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8841	4420	3.03	0.055
Error	71	103541	1458		
Total	73	112382			

189mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	37	85.42	49.62	8.16
Arg/Pro	31	106.01	42.73	7.67
Pro/Pro	6	101.6	55.7	22.7

Analysis of variance of erythema induced by 189mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	7408	3704	1.65	0.198
Error	71	158910	2238		
Total	73	166318			

238mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	37	115.17	51.98	8.55
Arg/Pro	31	136.43	38.91	6.99
Pro/Pro	6	135.4	53.2	21.7

Analysis of variance of erythema induced by 238mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	8235	4117	1.86	0.163
Error	71	156860	2209		
Total	73	16509			

300mJ per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	37	145.40	44.44	7.31
Arg/Pro	31	162.42	37.81	6.79
Pro/Pro	6	149.2	45.9	18.7

Analysis of variance of erythema induced by 300mJ per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	4976	2488	1.42	0.249
Error	71	124506	1754		
Total	73	129483			

Group 1 results – discussion

At none of the doses of UVR examined was there any significant difference between any of the genotypes and level of UV-induced erythema. This study group is similar in size to that studied by McGregor *et al* to investigate the risk of sunburn (74 compared to 78). The measurement of erythema by reflectance spectrophotometry as used here gives a numeric value for sensitivity to UVR, which is not dependent on the human eye for judgement, therefore is likely to be more accurate and reproducible than use of the minimal erythemal dose to determine UVR sensitivity.

Power calculations were carried out in group 1 using n=25 (balanced) and n=6 (least balanced). Between variance values at 119mJ were 813, while the within variance value was 651. Power was 1 when n=25, and 0.89 when n=6, the true power therefore lying between 0.89 and 1, which is good. At 300mJ, the between variance was 2488 and the within variance 1754. When n=25, power was 1, and 0.92 when n=6. Again these are good levels of power, and the p values obtained from the ANOVA tests can be accepted with confidence.

Erythema induced by incremental doses of UVR by p53 codon 72 genotype in group 2.

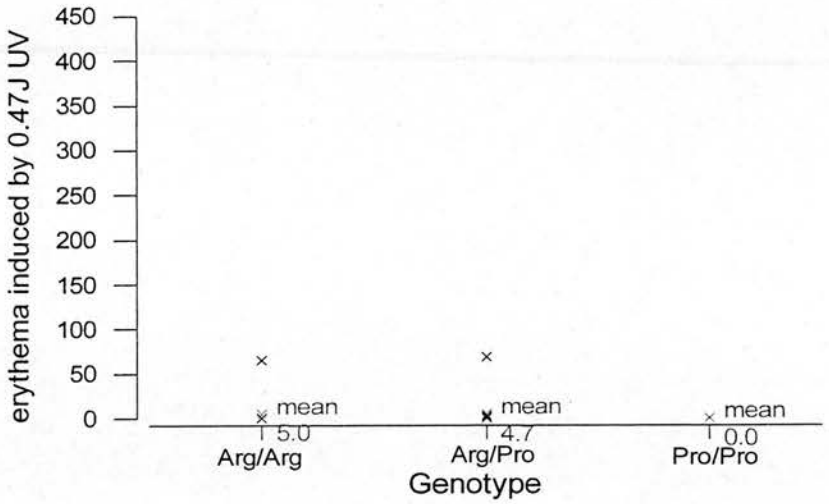


Figure 226 Erythema induced by 0.47J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

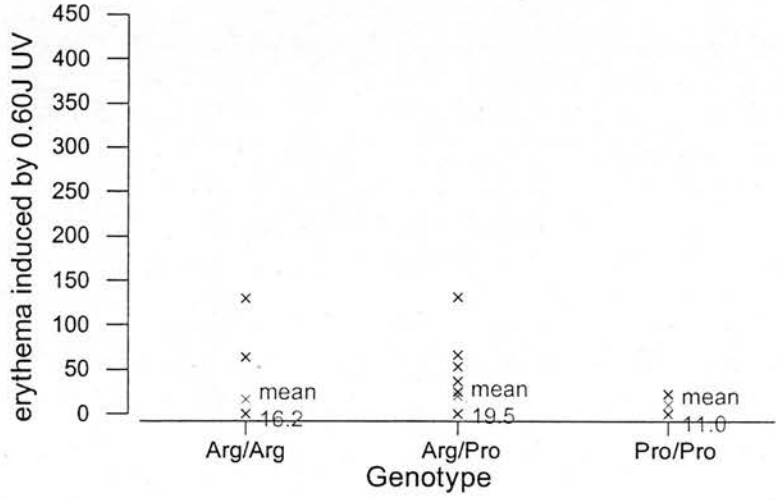


Figure 227. Erythema induced by 0.6J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

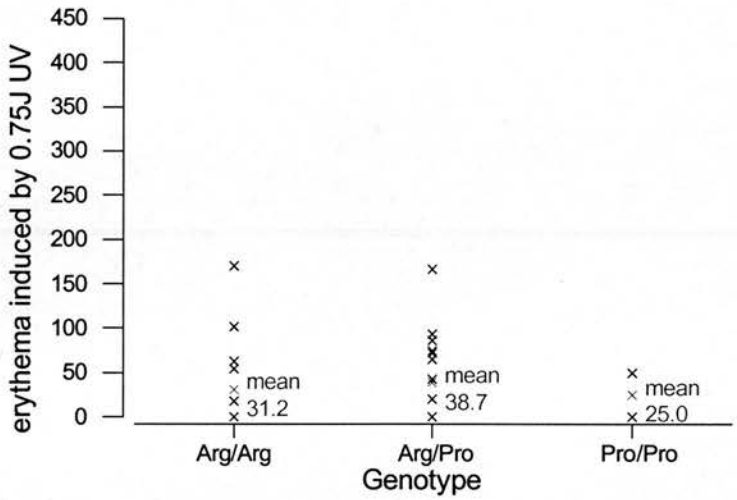


Figure 228 Erythema induced by 0.75J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

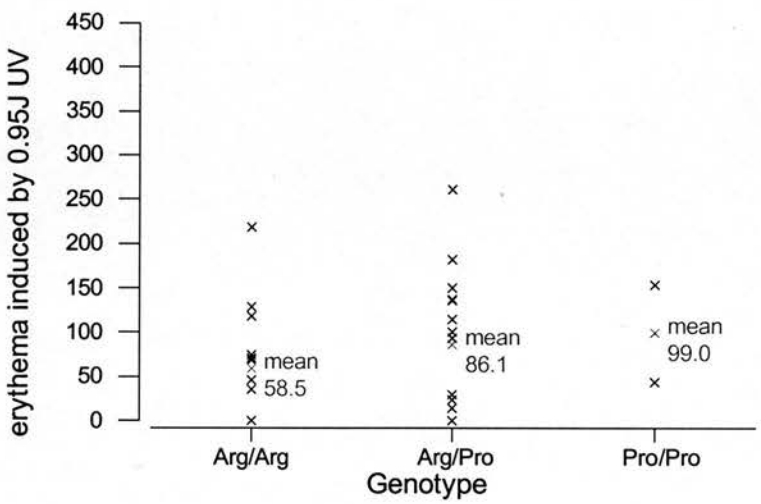


Figure 229 Erythema induced by 0.95J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

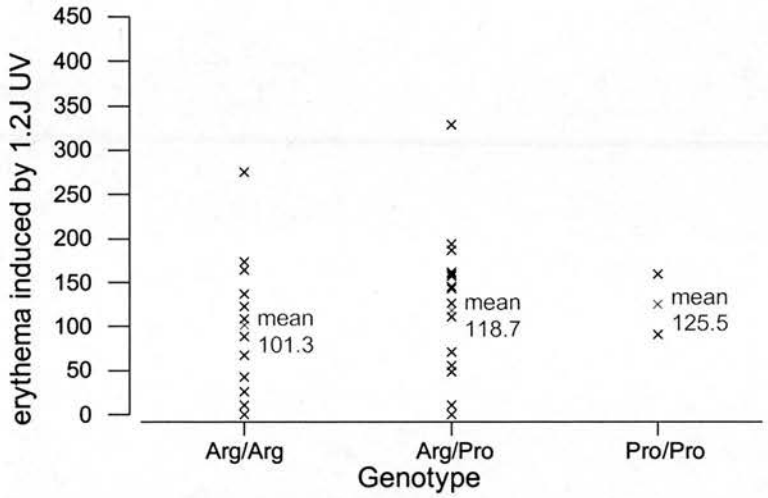


Figure 230. Erythema induced by 1.2J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

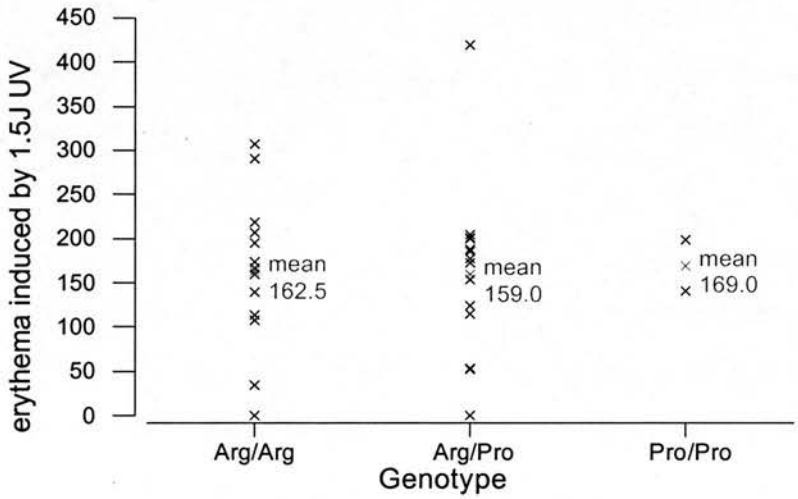


Figure 231 Erythema induced by 1.5J per cm^2 UV by p53 codon 72 genotype.
UV on inner forearm, measured at 24 hours

Analysis of p53 codon 72 genotype and erythemal response to UVR for each dose in group 2.

0.47J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	5.00	18.03	5.00
Arg/Pro	16	4.69	16.93	4.23
Pro/Pro	2	0.00	0.00	0.00

Analysis of variance of erythema induced by 0.47J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	44	22	0.08	0.927
Error	28	8201	293		
Total	30	8246			

0.6J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	16.2	38.6	10.7
Arg/Pro	16	19.54	36.77	9.19
Pro/Pro	2	11.0	15.6	11.0

Analysis of variance of erythema induced by 0.6J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	172	86	0.06	0.939
Error	28	38384	1371		
Total	30	38555			

0.75J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	31.2	53.0	14.7
Arg/Pro	16	38.7	49.6	12.4
Pro/Pro	2	25.0	35.4	25.0

Analysis of variance of erythema induced by 0.75J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	596	298	0.12	0.891
Error	28	71811	2565		

Total 30 72408

0.95J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	58.5	66.0	18.3
Arg/Pro	16	86.1	79.3	19.8
Pro/Pro	2	99.0	77.8	55.0

Analysis of variance of erythema induced by 0.95J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	6669	3335	0.61	0.550
Error	28	152680	5453		
Total	30	159349			

1.2J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	101.3	76.5	21.2
Arg/Pro	16	118.7	86.2	21.6
Pro/Pro	2	125.5	48.8	34.5

Analysis of variance of erythema induced by 1.2J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	2565	1283	0.20	0.824
Error	28	184022	6572		
Total	30	186588			

1.5J per cm^2

Genotype	N	Mean	StDev	SE Mean
Arg/Arg	13	162.5	87.8	24.4
Arg/Pro	16	159.0	92.4	23.1
Pro/Pro	2	169.0	41.0	29.0

Analysis of variance of erythema induced by 1.5J per cm^2 UV between genotypes:

Source	DF	SS	MS	F	P
Factor	2	220	110	0.01	0.986

Error	28	222222	7937
Total	30	222442	

Power calculations were carried out for data at 119mJ and 300mJ, using n=10 (balanced) and n=2 (least balanced). The between variance value was 22 at 119mJ and the within variance was 293. This gave a power of 0.16 when n=10 and 0.06 when n=2. At 300mJ, the between variance was 110 and within variance 7937, which gave a power of 0.07 when n=10 and 0.05 when n=2. The power in group 2 is extremely low, and would need to be increased in order to confirm that no association exists between p53 codon 72 genotype and erythemal response, which could perhaps be achieved by increasing group size. However, the same trend is seen as in group 1, where power was good.

The results of this study investigating an association between p53 codon 72 polymorphism and sensitivity to UVR in two independent study groups with a range of UV doses do not support the earlier findings of McGregor *et al* (2002). Although in both groups the numbers of Pro/Pro homozygotes is small, and the errors wide, there is no evidence to support an increased sensitivity to UVR at either body site at any of the UV doses examined in individuals carrying the Arg allele. If anything, there tended to be a higher level of erythema in the Pro/Pro groups. The small number of Pro/Pro homozygotes is in line with the expected frequency in such a population, however, this contributes to the large errors seen in the statistical analysis. One way to solve this could be to genotype larger numbers of individuals, then irradiate an equal number of each genotype with UV and measure their response. This would no longer be a random mix of the population however, and population-sampling errors would come into force. That the codon 72 alleles are found at different frequencies throughout the world, correlating as they do with latitude, suggests that if the codon 72 polymorphism is not itself associated with UVR sensitivity, as this data implies, it is possible that an independent association exists with skin colour, which is not related to how the skin responds to UV. A biased study group with equal numbers of each genotype could also cause any background associations of yet unidentified factors to be mistakenly labelled as the effects of the codon 72 polymorphism.

Effect of polymorphisms of repair genes on sensitivity to UVR.

Polymorphisms in exons 6, 22 and 23 of the XPD gene have previously been reported to be associated with an increased risk of the development of malignant melanoma (Tomescu *et al*, 2002). The study presented here genotyped two, independent, study groups, one n=74, the other n=31, which were both larger than the population analysed by Tomescu and colleagues, of 28 patients and 33 controls. The data presented here shows no association between any of the polymorphisms of XPD and increased sensitivity to UVR, as measured by erythemal response. UVR is the major causative agent for the development of melanoma; therefore it is possible that a polymorphism which leads to increased risk of melanoma would also show increased sensitivity to UVR. In none of the data analysed, for both study groups, at any UVR dose examined, was there an association between any polymorphism of XPD and sensitivity to UVR. The low number of individuals studied by Tomescu *et al* indicated the association they report between polymorphisms of XPD and melanoma may be due to chance. That no association was seen between the XPD polymorphisms of exons 6, 22 or 23 and sensitivity to UVR is in agreement with another, larger study of the association of these polymorphisms and risk of melanoma (Winsey *et al*, 2000), where there was no evidence of any association of these polymorphisms and risk of melanoma. It is still possible, however, that the XPD gene is associated with sensitivity to UVR, and that this was not revealed as the causative polymorphism was not analysed here, nor in linkage disequilibrium with those polymorphisms that were studied. However, there is little evidence to justify the analysis of further XPD polymorphisms.

The ERCC1 gene exon 4 polymorphism was not seen to be associated with sensitivity to UVR at any UV dose examined in either of the two study groups. The analysis of the two, independent, study groups, both of which gave the same conclusion, indicates there is no evidence of the exon 4 polymorphisms being involved with determining sensitivity to UVR. This is in agreement with the study by Winsey *et al* (2002) which found no

association between the exon 4 polymorphism and risk of malignant melanoma. The phenotypic effect of no functional ERCC1 protein is thought to lead to an embryonic lethal phenotype in humans, therefore it was speculated that a polymorphism which leads to minor changes in the wild-type protein would show an association with UV sensitivity, as ERCC1 is crucial in the NER pathway that repairs UV-induced damage. This was not observed here.

Mouse studies have shown that the lack of functional ERCC1 protein in mice leads to a runted phenotype with severe liver abnormalities and death before three weeks of age (McWhir *et al*, 1993). It is therefore possible that ERCC1 has another, as yet unidentified, function, and that the exon 4 polymorphism might lead to an association with the phenotype of this.

The XPF gene produces a protein which acts in complex with the ERCC1 protein in the initial incision stage of NER. The polymorphism of exon 11 of the XPF gene was analysed for an association with sensitivity to UVR. No association was observed between this polymorphism and erythemal response in either study group at any UV dose, suggesting it is not involved with determining sensitivity to UVR.

The XPG gene is involved with the initial incision process of damaged DNA in NER, and is also thought to have an additional role in the repair of oxidative damage. UVR induces DNA damage, both directly, which is repaired by NER, and indirectly though the generation of oxidative stress. The exon 15 polymorphism of XPG was therefore of particular interest in analysing erythemal response. An association was observed between the polymorphism and erythemal response, with the GG genotype displaying greater sensitivity to UV than the CG or CC genotypes. Analysis of three additional snps in the XPG gene revealed the suggestion of an association between the rs4150265 polymorphism and erythemal response. This is located within the intron immediately preceding exon 15, and therefore cannot be a causative mutation, but could be in linkage disequilibrium with the exon 15 polymorphism. No association was observed between the other two snps and erythemal response, which were a greater distance from exon 15.

Further analysis of this polymorphism is warranted. A larger study could confirm the association observed here with fewer errors, while a functional study could investigate whether exon 15 is directly involved, or is a marker for the causative mutation. One way to do this would be to take skin biopsies of irradiated skin, as mentioned previously. The number of sunburn cells could then be histologically determined, the hypothesis being that if the polymorphism does cause increased sensitivity to UVR, then cells from individuals with the GG genotype should have a greater percentage of sunburn cells, which have undergone apoptosis as a result of DNA damage.

The XRCC1 exon 10 polymorphism was found to show no association with sensitivity to UVR as measured by erythemal response. The XRCC1 gene is involved with the BER pathway, which repairs DNA damaged by oxidative stress. The formation of UV-induced photoproducts, which are repaired by NER, might be likely to cause a greater immediate threat to the integrity of the genome than that induced by oxidative stress. It might therefore be possible that the XRCC1 gene is not crucial for the repair of UV-induced DNA damage.

XRCC3 has a role in recombination repair, and cells which are deficient in XRCC3 have an increased sensitivity to a variety of DNA mutagens, including UVR. No association was observed between the polymorphism in exon 7 of this gene and sensitivity to UVR. This polymorphism has also been analysed by Winsey and colleagues, who found there to an association between this polymorphism and risk of developing malignant melanoma. As previously mentioned, the used of cadavers as the control population in the study by Winsey *et al* could introduce some doubt as to the validity to this report. A large study, with a cohort of melanoma patients, and a healthy, living control population investigated for any association with melanoma and also for sensitivity to UVR as measured by erythemal response would go some way to resolving the finding here of no association between XRCC3 exon 7 and sensitivity to UVR, and the findings of Winsey *et al* that this polymorphism is associated with increased risk of melanoma.

Effect of polymorphisms in repair genes to anthralin sensitivity.

The repair gene polymorphism detailed above were also analysed for any association with flux response following topical application of anthralin, an inducer of oxidative stress. As previously mentioned, oxidative stress is repaired in the main by the BER pathway, of which XRCC1 is a key component. XPG is also thought to have an additional role in the repair of oxidative stress distinct from its role in NER. None of the other repair genes analysed have any known role in the BER pathway, therefore if there were to be an association between anthralin induced flux and one of these polymorphisms, it would be expected to be XRCC1 or XPG.

At none of the polymorphisms of the repair genes analysed in this study was there an association with flux response to anthralin. The polymorphisms examined might not be reflective of the genes as a whole for response to anthralin, but in the absence of further study there is no evidence to suggest that any of the repair genes, ERCC1, XPD, XPF, XPG, XRCC1 or XRCC3 are involved with the repair of oxidative damage induced by anthralin. To further investigate the effect of these polymorphisms on anthralin response, a greater number of individuals would need to be studied. The effect of these polymorphisms at the cellular level could also be investigated, by measuring the repair capacity of oxidative stress in cells from individuals with different genotypes of these polymorphisms.

The GSTT1 gene and sensitivity to UVR.

No association was observed between the GSTT1 null allele, which produces no functional protein, and UV sensitivity. This was in contradiction to a study published by Kerb *et al* (2002), who report that this polymorphism does lead to increased sensitivity to UVR. However, the measure of UV sensitivity by in the study by Kerb and colleagues was by determining the MED. The MED is limited in its accuracy for several reasons as mentioned earlier, including that it is a threshold measurement , and is open to error by its need for the human eye to determine UV-induced erythemal. Reflectance spectrophotometry, as used here, should be likely to give a more accurate measurement

of sensitivity to UVR. A larger study would determine whether the association reported by Kerb *et al* is due to the errors occurring when measuring sensitivity to UVR by MED.

The GSTT1 gene and sensitivity to anthralin.

The GSTT1 null polymorphism was also investigated for any influence on sensitivity to anthralin, a generator of oxidative stress. As an antioxidant, GSTT1 might limit damage caused by the topical application of anthralin to cellular DNA. In this study, however, no association was observed between GSTT1 genotype and the flux response to anthralin at a variety of doses. From this data, GSTT1 does not seem to be associated with the prevention of DNA damage induced by anthralin, although a larger study group is needed to confirm this.

The p53 codon 72 polymorphism and UVR sensitivity.

Much controversy exists over this polymorphism and its resulting effects on increased risk of a variety of various cancers. Many studies have shown no association between p53 codon 72 polymorphism and risk of developing a particular cancer, while others report it to confer a greater susceptibility to the development to other cancers.

In this study, no association was observed between codon 72 genotype and sensitivity to UVR, as measured by reflectance spectrophotometry for erythemal response. It has previously been reported that this polymorphism leads to an increased sensitivity to UVR (McGregor *et al*, 2002). However, in their study, McGregor and colleagues determined sensitivity to UVR by the MED. As discussed before, the MED is an inadequate measure of an individual's sensitivity to UVR, being open to error. The data reported here suggests no such association between the polymorphism at codon 72, and UVR sensitivity in a study of approximately equal size. The measurement of UV sensitivity is likely to have been measured more accurately here, suggesting that this result is indicative of the phenotypic response to the polymorphism, i.e. that it does not appear to confer sensitivity to UV.

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No Association Between p53 Codon 72 Polymorphisms and Erythemal Response

To the Editor:

McGregor and colleagues report an association between allelic variants at codon 72 of the p53 gene and sunburn and non-melanoma skin cancer, with Pro/Pro homozygotes having a higher minimal erythema dose (MED), i.e., less sensitive to ultraviolet radiation (UVR). A subsequent study in the Journal has also reported an association between allelic variants at this p53 codon and melanoma (with the Pro/Pro alleles being less at risk) (Shen et al, 2003). In the McGregor study, both phototype and the MED were used as measures of UVR sensitivity. The finding of an association between allelic variants of the p53 gene and acute UVR sensitivity is important, as it may provide insight into the mechanism underpinning the reported association with skin cancer.

We have recently had the opportunity to examine the same p53 variants and UVR sensitivity, measured not as in the McGregor study by a threshold measurement, the MED, but by objective reflectance spectrophotometry, at a range of UVR doses on a different body site on each of two patient groups. Our results do not support the findings of McGregor et al (2002).

In group 1, 74 healthy residents of Edinburgh, UK, were irradiated on the lower back using a range of UVB doses (119–300 mJ per cm^2) from a lamp unit designed by Prof Brian Diffey (Regional Medical Physics, Newcastle, UK), containing a PL-S 9W/12 miniature flourescent lamp (Philips, Eindhoven, The Netherlands). In group 2, 31 psoriasis patients, prior to phototherapy, were irradiated on the inner forearm with a similar luminaire with six doses of UVB from a TL01 Philips tube (0.47–1.5 J per cm^2). Erythema was measured at 48 h in group 1 and at 24 h in group 2 with readings taken in triplicate. (Erythema measured at 24 h in group 2 to minimize disruption to patients' routine care.)

Baseline measurements were also taken on adjacent unirradiated skin and UVR-induced erythema defined as the increase in reflectance from the baseline values. Volunteers were phototyped according to the classification used by McGregor. Skin type breakdown was as follows: volunteers: 61 (I/II), 12 (III/IV), 1 (V/VI); psoriasis patients: 8 (I/II), 11 (III/IV), 0 (V/VI). Note this population group appears more homogeneous in terms of ethnic mix than those studied by McGregor.

Genotyping of the p53–72 polymorphism was carried out by PCR and restriction fragment length polymorphism analysis, using the primers 5′-ATC TAC AGT CCC CCT TGC CG-3′ and 5′-GCA ACT GAC CGT GCA AGT CA-3′ in a PCR reaction consisting of an initial melting step of 941C for 4 min, followed by 35 cycles of 941C for 40 s, 561C for 30 s and 721C for 30 s. The resulting 296 base pair (bp) fragment was digested overnight at 601C by BstUI. Digestion products were analyzed by electrophoresis through a 2% agarose gel stained with ethidium bromide. The Arg allele contains a BstUI restriction site, yielding two fragments of 169 and 127 bp. The Pro allele was not cut, and had a single band of 296 bp. The heterozygous genotype (Arg/Pro) gave three bands (296, 169, and 127 bp).

The results expressed as means and SEM for the various doses for the two study groups are shown (Tables I and II). Although like the McGregor study the number of Pro/Pro homozygotes is small, and errors wide, we found no

Table I. Mean erythema values per p53 codon 72 genotype group 1, n = 74 UVB on lower back with lamp unit containing a Phillips 9w/12 tube. Erythema measured at 48 h.

	Mean erythema ± SEM		
Dose (mJ per cm^2)	Arg/Arg (n = 37)	Arg/Pro (n = 31)	Pro/Pro (n = 6)
300	145.4 ± 7.31	162.42 ± 6.79	149.2 ± 18.7
238	115.17 ± 8.55	136.43 ± 6.99	135.4 ± 21.7
189	85.42 ± 8.16	106.01 ± 7.67	101.6 ± 22.7
150	45.61 ± 5.77	68.44 ± 7.65	58.9 ± 12.5
119	18.81 ± 3.77	28.62 ± 5.31	22.97 ± 6.07

SEM, standard errors of the mean.

Table II. Mean erythema values per p53 codon 72 genotype group 2, n = 31 UVB inner forearm with TLO1 Phillips tube. Erythema measured at 24 h

	Mean erythema ± SEM		
Dose (J per cm^2)	Arg/Arg (n = 13)	Arg/Pro (n = 16)	Pro/Pro (n = 2)
1.5	162.5 ± 24.4	159.0 ± 23.1	169.0 ± 29.0
1.2	101.3 ± 21.2	118.7 ± 21.6	125.5 ± 34.5
0.95	58.5 ± 18.3	86.1 ± 19.8	99.0 ± 55.0
0.75	31.2 ± 14.7	38.7 ± 12.4	25.0 ± 25.0
0.60	16.2 ± 10.7	19.57 ± 9.19	11.0 ± 11.0
0.47	5.00 ± 5.00	4.69 ± 4.23	0.00 ± 0.00

Abbreviations: MED, minimal erythema dose; SEM, standard errors of the mean; UVB, ultraviolet B; UVR, ultraviolet radiation

evidence to support any increase in sun sensitivity at either body site at any dose examined. If anything, the erythema tended to be higher in the Pro/Pro group.

There may be a number of reasons—apart from chance—for these differences. The MED is both a threshold measure and is influenced by basal pigmentation. Its reading may therefore be biased by genetic ancestry, methodological problems that reflectance spectrophotometry with examination of a range of doses of irradiation circumvents. It is also well known that the various codon 72 alleles are not distributed uniformly throughout human populations around the world, implying an independent (and confounding) association with skin color. Not all studies have shown an association between p53 codon 72 polymorphism and cutaneous malignancies (Baastiens et al, 2001). In the absence of further study we believe there is little compelling evidence to implicate p53 codon 72 alleles with sensitivity to UVR.

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